D. USE OF BIOLOGICAL RESPONSES TO SMOKE AND SMOKE COMPONENTS AS INDICATORS OF SMOKE INTAKE

Cigarette smoke is known to elicit a multitude of biochemical and physiological responses. It is conceivable that one or more of these responses might be useful as a quantitative indicator of smoke intake and, therefore, a review of biological responses was considered worthy of inclusion in this study. Responses could, for example, be manifestations of changes in enzyme levels, tolerance to or exaggeration of certain pharmacological effects, and many other forms of biological adaptations. To be useful as a measure of smoke intake, the ideal response should have some of the same requirements as have already been cited for ideal chemical markers of smoke intake. A useful biological response would have to possess at a minimum the following:

- Specificity -- the response must be caused only by smoke inhalation and not be appreciably altered by other factors such as diet and environment.

- Proportionality -- within an individual and to a somewhat lesser extent, among individuals the amount of smoke intake should cause a predictable and quantitative response.

- Persistence -- appropriate duration of the response. The response should persist long enough to allow time to measure or record the event, yet not so long that it cannot reflect a major change in smoke intake.

- Minimal Variability between Individuals -- the response to an amount of smoke intake should be similar between individuals.

Table 3 lists the compounds reported in smoke, the species in which biological responses were observed, as well as a brief description of the biological response itself.

Our review of the literature on the biological effects of smoking has turned up very few instances in which the response to smoke has been shown to be directly applicable to quantitative estimation of smoke intake, since most of the research has involved the qualitative aspects of smoking.

In attempts to correlate the various biological responses with smoking, most of these studies have used smokers vs. non-smokers to determine the effects of smoking (chronic) or else they involved studies to determine some acute response during or immediately after smoking. In the absence of suitable methods for estimating actual smoke intake, most of the correlations were based on a subjective estimation of smoke intake or habit. To what extent poor correlations between various reported biological responses and smoke intake are related to inadequate knowledge of retained dose cannot be ascertained. A brief discussion

ABBREVIATIONS USED IN TABLE 3

↑	Increases, higher
↓	Decreases, lower
→	Leads to, causes
COHb	Carboxyhemoglobin
BUN	Blood urea nitrogen
SGOT	Serum glutamic oxalic transaminase
SGPT	Serum glutamic pyruvic transaminase
LDH	Lactic dehydrogenase
HR	Heart rate
BP	Blood pressure
NEFA	Non-esterified fatty acid
FFA	Free fatty acid
FA	Fatty acid
5-HT	5-Hydroxytryptamine
NE	Norepinephrine
EPI	Epinephrine
5-HIAA	5-Hydroxy-3-indole acetic acid
ADP	Adenosine diphosphate
FEV	Forced expiratory volume
SV	Stroke volume
ADH	Antidiuretic hormone
OHCS	Hydroxycorticosteroids
KA units	King Armstrong units
pCi	pico Curies

TABLE 3

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Nicotine (4 µg/kg/min IV)	Hall (201)	Cat	Nicotine (IV) → ↑ release of norepinephrine into the effluent from the perfused third cerebral ventricle when compared against control (saline IV) over the half-hour of the test and for 10-20 min. after the infusions were stopped.
Smoke	Butkus (76)	Man	In an acute study of smokers and non-smokers, smoking had no significant effects on the serum free cholesterol, phospholipid, or triglyceride fraction. Smoking and non-smoking tests were carried out on the same subjects after a 12 hr. fast and abstinence from smoking, with subjects serving as their own control.
Smoke (Chronic)	Stocksmeier (513)	Man	In a limited study of 139 patients with myocardial infarct, no definite statistical differences among those who stopped smoking (90), those who did not (34), and non-smokers (15) could be shown. However, some trends seemed evident: a) A higher index of re-infarct in smokers who did not stop after infarct. b) For the entire group blood pressure, body weight, blood cholesterol and triglycerides declined at 2.5 months after infarct. c) In smokers who did not stop smoking, blood pressure did not change, body weight ↑, blood cholesterol and triglycerides did not change 2.5 months after infarct.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Higgins (214)	Man N=8641	Smokers compared to non-smokers showed the following changes:

	Men	Women
Weight	↓*	↓*
Height	---	---
Serum cholesterol	↑	---
Systolic pressure	↓	↓*
Heart rate	↑*	---
F.E.V.$_{1.0}$	↓*	↓*
Incidence of:		
Bronchitis	↑*	↑*
Emphysema	↑	---
Peptic Ulcer	↑	↑*
Psychoneurosis	---	↑*
Coronary heart disease	---	---
Asthma	---	---

--- No Change

*Statistically significant ($p < 0.05$)

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Kershbaum (265)	Man N=8	a) Smoking four cigarettes → ↑ rate of *in vitro* cholesterol crystallization* in serum of all 8 subjects.
		Dog N=6	b) Nicotine (0.9 mg/kg IV) → significant ↑ in cholesterol crystallization *in vitro*.
Smoke	Modzelewski (341)	Man N=102 smokers 20 age-matched non-smokers	a) Blood levels of cholesterol, β-lipoproteins, and FFA were not significantly different from control in subjects smoking < 10 cigarettes per day.
			b) Cholesterol, β-lipoproteins, and FFA were significantly higher in subjects smoking > 10 cigarettes per day.
			c) Differences were more pronounced between control and heavy smokers > 30 years old.
Smoke (Nicotine)	Doyle (144)	Man	a) Nicotine → ↑ HR, ↑ cardiac output ↓ cutaneous blood flow, and ↑ coronary blood flow in normal subjects.
			b) Smaller or no ↑ in coronary blood flow is seen in patients with coronary heart disease.
			c) Serum FFA ↑.
			Conclusion: nicotine → ↑ epi → ↑ FFA.

* A measure of the effect of serum lipid extracts on the rate of crystallization of cholesterol from a supersaturated cholesterol-triglyceride solution.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Nicotine (1 cigarette)	Levine (299)	Man	a) Platelet aggregation is enhanced by smoking one cigarette. b) Free fatty acid ↑ in plasma. c) Lettuce cigarettes and sham smoking caused no change in (a) or (b). d) Nicotine → epinephrine release, → ↑ FFA, and ↑ platelet aggregation.
Smoke	Ciampolini (99)	Man	Compared to non-smokers, smoking one cigarette → ↑ (significant) non-esterified FA, ↑ (non-significant) FFA, ↑ (significant) cholesterol in the plasma.
Smoke	Kershbaum (262) (261)	Man	a) Cigarette smoking → ↑ catecholamine excretion; ↑ catecholamine → ↑ FFA, triglycerides. b) Cigar and pipe smoke → effects similar to cigarette smoke if inhaled. c) Nicotine (IV) → } Smoke → } → ↑ catecholamine excretion.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Cristaldi (117)	Man	a) Basal values of plasma N.E.F.A. are generally higher in patients with coronary disorders than in normal subjects. b) Cigarette smoking → ↑ N.E.F.A. in both normal subjects and patients with coronary disorders, but the ↑ in coronary patients is more pronounced and more protracted.
Smoke	Boyle (64)	Man (N=1,104)	a) β-lipoprotein and cholesterol in serum ↑ with age in both smokers and non-smokers. b) β-lipoprotein and cholesterol concentrations in serum were higher in smokers than non-smokers -- smokers having levels comparable to non-smokers 5-7 years older. c) β-lipoprotein and cholesterol levels are correlated $r = 0.84$ ($p < 0.001$) to each other and to percent overweight.
Smoke (Chronic)	Sackett (467)	Man	a) The finding of calcification of the thoracic aorta on chest X-ray was determined to be a valid indication of marked aortic atherosclerosis by later post-mortem findings. b) Smokers compared to non-smokers have a higher incidence of calcification of the thoracic aorta in age groups over 60 years being highly significant for age group 70 and over.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Gross (194)	Man	Smoking one cigarette → the following serum changes: a) ↑ cholesterol b) ↑ sebacic acid ester c) ↑ β-lipoproteins d) ↓ glucose e) ↓ free glycerol f) Smoking → no change in total glycerol or triglycerides.
Smoke	Caganova (80)	Man (49 students)	Compared to non-smokers, smokers have significantly elevated serum levels of cholesterol, iodine number, β-lipoproteins, and β/α-lipoprotein index.
Smoke	Pincherle (427)	Man (7000 executives)	a) Smokers have ↑ levels of serum cholesterol compared to non-smokers of the same age, but the difference is small and not significant. b) Serum cholesterol correlates positively with obesity, stress and blood uric acid.
Smoke	Richard (447)	Man	A weak positive correlation exists between tobacco consumption and blood cholesterol when age and weight are adjusted for.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Wille (566)	Man	a) The correlation coefficients between smoking and blood cholesterol and blood cholesterol and age were significant. b) The correlation coefficient between pre-β-lipoproteins and smoking was not significant.
Smoke	Schwartz (Cited in 531)	Man	a) Positive correlation between blood cholesterol and smoking habits. b) Positive correlation between blood cholesterol and body weight. c) Smoking and body weight correlate negatively so as to possibly mask the correlation between smoking and blood cholesterol.
Smoke	Romslo (Cited in 531)	Man	Smoking correlates with ↑ blood triglycerides but not significantly.
Smoke	Burney and Enstein (Cited in 531)	Man	Blood lipids did not correlate with smoking.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Pozner (432)	Man N = 47	Significance of the Difference of the Means (p Values) in Smokers and Non-Smokers

Direction of Change Smokers minus N.S.		Women	Men
No. of subjects		16	31
Plasma turbidity	+	N.S.	N.S.
*Stypven-times	-	N.S.	N.S.
Fibrinolysis-times	-	N.S.	N.S.
Phospholipids	+	0.002	N.S.
Total cholesterol	+	0.001	N.S.
Free cholesterol	+	0.010	N.S.
Ester cholesterol	+	0.010	N.S.
Triglycerides	+	0.10	N.S.
β-lipoproteins	+	0.01	N.S.
Pre-β lipoproteins	+	N.S.	0.001
α lipoproteins	+	N.S.	0.001

*Measure of clotting time.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Finley (163)	Man	In smokers compared to non-smokers analysis of returns from pulmonary lavage show: a) ↓ Surface active material (surfactant). b) ↑ Alveolar macrophages.
	Clements (102)		From the data in the above paper, author calculates that the surface active lipids of smokers are contained in the cellular fraction.
Smoke	Claude (100)	Man N = 676	There is a significant positive correlation of blood FFA with smoking, BP, and with blood levels of insulin, triglycerides, and cholesterol, but not with body build.
Smoke (Chronic)	Dalderup (125)	Man	a) Fibrinolysis is definitely inhibited by medium to heavy smoking. b) Heavy smokers with low lipids in the large particle fraction of serum, have longer fibrinolysis times when compared to non- or light smokers.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Pozner (432)	Man N = 64	Plasma clotting time (Stypven-times) and fibrinolysis times were not significantly different between heavy smokers and non-smokers, although they were decreased in smokers.
Smoke (Chronic)	Menon (333)	Man	Smokers had a decreased euglobulin lysis time (index of fibrinolysis) compared to non-smokers, but the difference was not statistically significant.
Smoke (Chronic)	Hawkins (208)	Man	Compared to non-smokers, smokers had: a) ↑ platelet activity (clumping in presence of ADP) b) ↓ coagulation time c) ↑ tensile strength of clot.
Nitrogen Dioxide	Rowlands (460) (459)	Mouse Rabbit	Results of *in vivo* exposure to NO_2 or smoke imply: a) Agents migrate through the erythrocyte membrane and, b) may destructively interact with hemoglobin by removing the heme iron and forming two types of non-heme iron complexes.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Nitric Oxide	Gibson (177)	Sheep	Hemoglobin (more specifically the heme portion) has a remarkably high affinity for nitric oxide *in vitro* in the order of 1500X greater than carbon monoxide as determined by reversion spectroscopy. This affinity of NO for Hb is chiefly due to the slow rate of breakdown of NOHb. The half time for dissociation of the first NO molecule from $Hb_4(NO)_4$ at 19°C was ∿ 8 hrs at pH 9 and ∿ 3 hrs at pH 6. The velocity constant for the reaction, under standard conditions of temperature and pH, varied from sheep to sheep presumably owing to individual differences in the globin portion of the hemoglobin molecule.
Smoke	Nilsson (382)	Man	In smokers C-reactive protein was generally ↑ and was correlated with number of cigarettes smoked.
Smoke	Kreis (277)	Man	a) A precipitation reaction was demonstrated in human sera to which a soluble extract of tobacco was added. b) No relationship between smoking habits of serum donors and the precipitation reaction could be shown.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Esber (157)	Mice	a) Mice exposed to total smoke or gas phase from smoke for 1-7 days had a suppressed immune response. Return to normal immune response took around 14 days after last exposure. b) Suppression occurred with a simultaneous (or a 2-day delayed) exposure of smoke and an antigenic stimulus. c) Prolonged exposure of 14 or 21 days did not further suppress immune response.
Smoke	Keal (256)	Man	a) In early bronchitis no relationship is apparent between sputum analysis for neuraminic acid and smoking habits. b) In late bronchitis neuraminic acid values appear to ↑ with number of cigarettes smoked.
Smoke	Kosmider (275)	Man	a) Serum of smokers → ↑ precipitating titre of immunoglobin IgM and IgA and ↓ titre of immunoglobin IgG and isoagglutinin anti-A. b) Saliva of smokers → ↑ precipitating titre of immunoglobin IgA and IgG. Authors speculate differences in smokers and non-smokers are due to chronic antigen stimulation, both systemic and local, as a consequence of concomitant inflammation of the airways.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Kershbaum (264)	Man N=9 Dog N=6 Rat N=25	Four cigarettes in man within one-half hour, 0.9 mg/kg IV nicotine in dogs, and 0.5 mg/kg nicotine IP in rats all → ↑ in 11-hydroxycorticosteroids of 68%, 86% and 63% respectively.
Smoke	Tucci (529)	Man N=75	There was no statistically significant difference between smokers and non-smokers in their urinary levels of 17OHCS, 11-OHCS, cortisol, epinephrine, or norepinephrine.
Smoke (Acute)	Hendry (210)	Man	Smoking → ↑ ADH → ↓ water output in urine.
Smoke	Granerus (183)	Man	a) Smoking → ↑ excretion of methylimidalzole acetic acid in urine from 2.3 mg (non-smokers) to 3.1 mg for smokers. b) Highest values corresponded to the largest cigarette consumption. c) No significant difference in methylhistamine excretion between smokers and non-smokers.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Nicotine	Bishun (53)	Mouse *(In vivo)*	a) 0.07-0.09 µg nicotine/total body weight → ↑ incidence of chromosome aberrations in leukocytes.
		Man *(In vitro)*	b) Nicotine is cytotoxic to leukocytes at 2 µg/ml for brief exposures and at 0.5 µg/ml for exposures of 48 hours.
Smoke	Schievelbein (475)	Man N = 31	a) By t test analysis smokers had higher urinary excretion of 5-hydroxy-3-indole-acetic acid (5-HIAA) than non-smokers.
			b) Non-smokers excreted significantly more 5-HIAA after smoking cigarettes.
			c) Some smokers had very high levels of 5-HIAA.
Smoke (Acute)	Czarnik (122)	Woman	a) Smoking → significant transient ↑ in 5-HT levels in sera.
			b) 5-HT levels in smokers prior to smoking not statistically different from levels in non-smokers.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Degwitz (134) Hopsu (228)	Man	a) Urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA) is not elevated in smokers compared to non-smokers. b) Urinary excretion of 5-HIAA did not increase in non-smokers after they smoked 6-10 cigarettes.
Smoke (Chronic)	Kolber-Postepska (271)	Man (N = 14)	a) Urinary excretion of 5-HIAA was higher but not significantly in smokers compared to non-smokers on a per 24 hr. basis. Conditions and age were about the same for both groups. b) During the hours of 3 p.m. to 11 p.m. smokers excreted much more 5-HIAA than non-smokers.
Smoke (Acute)	Chiancone (92)	Man	An approximately linear ↑ in urinary elimination of tryptamine/24 hrs. compared with the number of cigarettes smoked between 10 and 40 cigarettes.
Smoke (Acute)	Alperovitch (4)	Man	Smoking → no change in urinary levels of cynurenin, N-1-methyl nicotinamide, and anthranilic acid which may indicate that smoking does not affect tryptophan metabolism.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Brown (71)	Man	There is no statistically significant difference in the urinary excretion of tryptophan or niacin metabolites in urine of non-smokers, in active smokers, or in smokers who have stopped smoking for 3 weeks.
Smoke	Nishimura (384)	Man	a) Heavy smokers show a higher incidence of Compound IV (believed to be cinnabarinic acid) in urine than non-smokers. b) ↑ levels of Compound IV are found in patients with bladder tumors. c) 1.5 gms/day of ascorbic acid (an antioxidant) has been shown to eliminate Compound IV from urine of both normal and heavy smokers, and patients with bladder tumors.
Smoke	Linnell (306)	Man	a) Smokers have ↓ levels of serum B_{12} (444 µµg/ml) compared with 472 µµg/ml for non smokers. b) Smokers have ↑ excretion of B_{12} in urine (81.2 mµg/24 hrs) compared with 60.3 mµg/24 hours for non-smokers. c) Smokers have ↑ excretion of SCN^- in urine (207.8 µ moles/24 hrs) compared with 90.5 µ moles/24 hours for non-smokers. Author suggests that ↓ levels of B_{12} in serum causes ↓ ability to detoxicate CN^- via this vitamin, so detoxification by SCN^- pathway ↑.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (SCN^-)	Courant (116)	Man	a) No statistical difference in pH or peroxidase activity of smokers vs. non-smokers saliva. b) Antibacterial activity against *Lactobacillus acidophilus* significantly ↑ in smokers.
SCN^-	Bourke (61)	Man	Thiocyanate was found to be a non-specific activator of the thyroid.
Smoke	Foulds (166)	Man	a) Serum B_{12} levels in tobacco amblyopes were significantly ↓ (196 μμg/ml) compared with non-smokers (237 μμg/ml). b) 40% of tobacco amblyopes showed ↓ serum levels of B_{12} (150 μμg/ml and below) and 45% had defective B_{12} absorption. c) Serum B_{12} levels were similar in non-smokers and non-amblyopic smokers. d) Direct relationship exists between B_{12} absorption, serum B_{12} levels, and tobacco consumption in the development of tobacco amblyopia.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (CN^-) (SCN^-)	Wilson (570)	Man	a) Plasma SCN^- levels in smokers were much ↑ than non-smokers, while plasma CN^- levels were higher but not statistically significant. b) Total serum B_{12} similar in smokers and non-smokers. c) Hydroxocobalamin, the difference between non-CN-extracted B_{12} and total B_{12}, is significantly smaller in smokers than non-smokers. d) Non-CN-extracted B_{12} as a percent of total B_{12} is significantly higher in smokers than non-smokers. e) There was an inverse relationship between SCN^- concentration and the difference between total serum B_{12} and non-CN-extracted B_{12}.
Smoke	Pettigrew (417)	Man	a) Levels of reduced glutathione (G.S.H.) were significantly ↓ in red cells of patients with tobacco amblyopia. b) GSH believed to change CN^- to SCN^-. B_{12} helps to elaborate GSH from oxidized glutathione.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (CN^-)	Wells (556)	Man	a) Smoking (CN^-) may → amblyopia and other neurological disorders, especially in cases of B_{12} deficiency. b) Plasma SCN^- levels were uniformly ↑ in smokers than non-smokers. c) In smokers with neurological disease due to B_{12} deficiency, SCN^- levels tended to be ↓ than controls. d) In non-smokers with neurological disease due to B_{12} deficiency, SCN^- concentration were not significantly different from control.
CN^-	Lindstrand (305)	Man	a) One of the forms of B_{12} (hydroxocobalamin) may be responsible for detoxification of exogenous CN^-. b) Author states if the following is true: $\underset{\text{Total } B_{12}}{A} = \underset{\text{Cyanocobalamin}}{B\ CNB_{12}} + \underset{\text{Hydroxocobalamin}}{C\ OHB_{12}}$ then (A-B) or A/B could be inversely related to amount of smoking. c) Author's results not conclusive.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Dastur (129)	Man	a) The serum Vitamin B_{12} levels determined in four groups decrease in the order: non-vegetarian non-smoker, non-vegetarian smoker, vegetarian non smoker, and vegetarian smoker. b) Thiocyanate is higher in smokers than non-smokers.
Smoke	Pelletier (411) (412)	Man	a) Smokers have significantly ↓ plasma and blood Vitamin C levels (0.3 mg%) compared to 0.7 mg% for non-smokers. b) Load doses of Vitamin C → ↑ retention and ↓ urinary excretion in smokers compared to non-smokers. c) At doses of 58-98 mg/day of Vitamin C smokers excrete 40% less Vitamin C than non-smokers. d) Indicates less Vitamin C is effectively available for utilization by smokers, or that smokers are utilizing Vitamin C differently.
Smoke	Brook (67)	Man	a) Cigarette smoking → significantly ↓ Vitamin C concentrations in leukocytes from 24.6 (non-smokers) to 18.8 mg/10^8 white cells in smokers. b) Smoking → significantly ↓ plasma Vitamin C levels from 0.62 mg% (non-smokers) to 0.44 mg% for smokers.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Loh (309)	Man	Leukocyte and plasma ascorbic acid levels are consistently ↓ in smokers when compared to non-smokers.
Polycyclic Hydrocarbons	Wiebel (562)	Mice	a) Aryl hydrocarbon [benz(a)pyrene] hydroxylase (AHH) inducibility varies from tissue to tissue in a given strain. b) In liver resistant strains, injection or topical application of benz(a)anthracene can induce AHH in extra hepatic tissues such as lung, kidney, skin, or small intestine.
Nitrogen Dioxide (5-50 ppm) Ozone (0.75-10 ppm)	Palmer (404)	Rabbit	a) NO_2 did not depress benzpyrene hydroxylase activity in tracheo-bronchial mucosa. b) O_3 did depress benzpyrene hydroxylase activity. Enzyme is believed to inactivate the carcinogenic potential of polyaromatic hydrocarbons.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Welch (555)	Rat	a) Smoking → production of an inducible enzyme, aryl hydrocarbon hydroxylase (AHH; also called benzpyrene hydroxylase). b) Highest levels of inducible enzyme found in lung and placenta. c) Metabolism of acetophenetidin ↑ due to prior exposure to smoke. d) Metabolism of acetophenetidin ↑ due to prior exposure to 3-methylcholanthrene.
Smoke	Kellerman (259)	Man	Highly significant difference in aryl hydrocarbon hydroxylase inducibility was seen in: Heavy smokers with cancer N = 50 { 30% high inducibility, 66% intermediate Healthy controls N = 85 { 9.4% high inducibility, 45.9% intermediate

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Chronic)	Pantuck (405) (406)	Man	a) Plasma phenacetin levels following a 900 mg oral dose were significantly ↓ in smokers than non-smokers.
			b) Plasma levels of phenacetin's major metabolite, N-acetyl-p-aminophenol (APAP), were ↓ in smokers, but not significantly.
3,4 Benzpyrene		Rat	c) In rats previously dosed with 3,4 benzpyrene (3,4 BP), the concentration of phenacetin following IP injection was significantly ↓ in whole body homogenates than in controls.
			d) An *in vitro* culture of intestinal mucosa of rats previously dosed with 3,4 BP, transformed phenacetin to APAP at a ↑ rate compared to cultures from control rats.
			Conclusion: smoke, more specifically 3,4 BP → ↑ the rate of metabolism of phenacetin. That plasma levels of APAP are not changed is consistent with the results in (d) which indicate that phenacetin is metabolized in part by the intestinal mucosa before it enters the blood stream.
Smoke (Acute)	Unghvary (530)	Man	One cigarette → appearance of fluorescent substances in blood (different from catecholamines) which are maximum at 5 minutes and disappear completely in one hour.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Acute)	DaSilva (128)	Man N = 21 (Smokers and non-smokers usually pooled)	Smoking one cigarette → significant a) ↑ airway resistance by 10.93%. b) ↓ mean expiratory flow by 4.15%. c) In smokers with chronic bronchitis ↓ closing volume by 5.6%. d) In non-smokers no change in closing volume. e) No change in dynamic compliance, (i.e., not frequency dependent).
Smoke (Chronic)	Binns (52)	Monkey	Airway resistance increases from control, during and after chronic exposure to cigarette smoke. No mean values given.
Nicotine (SC injection) (1 mg/kg daily)	Tashkin (517)	Rat	a) Chronic dosing of 2 month old rats → ↓ compliance of lungs. b) Chronic dosing of 2 year old rats → no change in compliance of lungs.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Acute)	Diamond (137)	Man	a) In normal subjects -- smokers and non-smokers, no changes were observed after smoking one cigarette in forced vital capacity (FVC), the ratio of forced expiratory volume in one sec./FVC, or in mid-expiratory flow. b) Non-smokers showed a significant ↑ in mean expiratory resistance after one cigarette. c) Heavy smokers showed a significant ↓ in mean expiratory resistance after one cigarette.
Smoke (Acute)	Simonsson (492)	Man	In normal smokers and in a group of patients (all smokers) with chronic generalized obstructive, non-specific pulmonary disease, pulmonary function tests showed the following changes immediately after one cigarette:

	Normal	Patients
FEV_1	↓ ($p < 0.05$)	↓ ($p < 0.01$)
FEV%	↓ ($p < 0.05$)	↓ (n.s.)
FVC	0	↓ (n.s.)

FEV_1 = Forced expiratory volume in one second.

FVC = Forced vital capacity.

FEV% = FEV/FVC.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke	Camner (Cited in 531)	Man	Between monozygotic twins discordant with respect to smoking, smokers had significantly slower tracheobronchial clearance rates.
Smoke	Albert (Cited in 531)	Man	a) Mean bronchial clearance time was ↑ in smokers (170 min.) compared to non-smokers (126 min.). b) Cigarette smoking → diminished pulmonary clearance in upper airways first. c) As a result, mucus cleared from lower airways accumulated in the larger airways where stasis occurred.
Smoke	Ewert (158)	Man	a) Ciliated transport activity in outer nasal passages affected by smoking. b) Statistically significant ↓ flow rate for smokers (3.6 mm/min.) compared to 4.8 mm/min. for non-smokers at 43.6% relative humidity.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Acute)	Bang (26)	Man	a) Human nasal mucous flow rates were found to be very variable between smokers and non-smokers. b) No consistent change in flow rates was caused by exhaled cigarette smoke and some other environmental pollutants.
Smoke (Chronic)	Lamb (282)	Rat	a) Chronic exposure to diluted smoke → ↑ goblet cell density at all levels of the bronchial tree. b) The increase is highest in the trachea and diminishes further down the bronchial tree.
Nitrogen Dioxide (15 ppm)	Parkinson (407)	Rat	Morphological changes in the lung: a) Loss of normal height relationship between ciliated and non-ciliated bronchiolar epithelial cells. b) Denudation of cilia evident.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Smoke (Aqueous smoke extract)	York (576)	Sheep	Aqueous smoke extract applied to a cell culture of pulmonary macrophages: a) → ↓ rate of O_2 uptake (and it remains depressed). b) → ↓ viability of cells.
Smoke	Rylander (Cited in 531)	Guinea Pig	a) Acute exposure to smoke of 5 or more cigarettes → significant ↓ in number of alveolar macrophages. b) More prolonged exposure to cigarette smoke → ↑ in number of alveolar macrophages over control values.
Smoke	Dobisova (Cited in 531)	Man	No relationship found between smoking and composition of bacterial flora in respiratory tree.
SO_2 (0.01-1%) or Smoke	Cho (93)	Dog	a) SO_2 inhalation → bronchoconstriction with dilation of bronchial arteries. b) Smoke inhalation → bronchoconstriction with constriction of bronchial arteries.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Acrolein	Lyon (312)	Rat Guinea Pig Monkey Dog	a) 0.7 ppm (R.E.) → chronic inflammatory lung changes and occasional emphysema. b) 3.7 ppm (R.E.) → non-specific inflammatory changes in lung, liver, kidney. → squamous metaplasia and basal cell hyperplasia of trachea in dogs and monkeys. → squamous metaplasia of lung bronchi in monkeys. → wt. ↓ in all species. c) 0.22 ppm (C.E.) → moderate emphysema, acute congestions, focal vacuolization of the bronchial epithelial cells with ↑ secretory activity in dogs. d) At all dose levels there was no significant change in following serum biochemical determinations: (1) sulfobromophthalien (dogs) (2) alanine amino transferase (3) aspartate aminotransferase (4) liver alkaline phosphatase (5) liver tyrosine amino transferase (6) urea nitrogen e) All levels → signs of irritation, e.g., nasal discharge and lacrimation.

R.E. = Repeated exposure 8 hrs/day, 5 days/week.
C.E. = Continuous exposure 24 hrs/day for 90 days.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
Cadmium	Friberg (168)	Man	a) Chronic exposure (> 8 yrs) to Cd dust → pulmonary emphysema → anosmia → small ulceration of nasal mucosa → proteinurea → renal damage → mild anemia → acceleration of erythrocyte sedimentation rate → ↑ of α-globulin fraction in blood serum. b) In humans, inhaled Cd is demonstrable in substantial amounts mainly in the kidney, liver, pancreas and thyroid.
Furfural	Rice (446)	Rat Hamster	a) Causes liver cirrhosis in rats. b) Speculated to have carcinogenic effect on respiratory tract in conjunction with benzo(a)pyrene.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
CO (Smoke)	Astrup (18) Astrup (19)	Man	a) Elevated COHb causes an exaggerated shift to the left of the oxyhemoglobin dissociation curve. b) The shift may be caused in part by decreased 2,3-Diphosphoglycerate (DPG) in RBC's. c) CO may affect the levels of 2,3-DPG.
CO (Smoke)	Astrup (20)	Man	a) 10% COHb levels are occasionally reached in smokers. b) This level causes a leftward shift of the oxyhemoglobin dissociation curve.
Smoke (5 cigs/1 hour)	Sugitani (516)	Man 20 students	a) Heavy smokers showed a > 1% ↑ COHb and a noticeable ↑ in levels of blood nicotine. b) Light smokers showed < 1% ↑ COHb and no marked change in blood nicotine.
CO (Dosage)	Adams (1)	Dog	Linear ↑ coronary blood flow and linear ↑ HR as COHb ↑ up to 20% saturation.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
CO (Dosage)	Pirnay (428)	Man	a) 15% COHb → compensatory ↑ HR and ventilation during moderate exercise, but no change in 0_2 consumption. b) During maximal exercise, compensatory response to 15% COHb was inadequate → 15% ↓ 0_2 consumption.
CO (Dosage)	Shephard (490)	Man	2-3% COHb and 5% COHb → no significant change in HR compared to HR before expousre to CO.
CO (Dosage)	Paulson (409)	Man	20% COHb saturation: a) → Cerebral blood flow ↑ 26%. b) Jugular 0_2 % sat. ↑ 4.5%. c) → P_{50}* of 0_2 ↓ 4.3 mm Hg. d) No change in PCO_2 or pH. e) No change in cerebral metabolic rate. } Compared against control

*P_{50} = PO_2 at 50% 0_2 saturation.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
CO (Dosage)	Eckardt (147)	Monkey	a) Two-year exposure to 20 or 65 ppm CO caused no significant pathophysiological changes in the following serum determinations: glucose, BUN, total bilirubin, total protein, albumin, Na^+, K^+, Cl^-, Ca^{++}, CO_2, SGOT, SGPT, and LDH. b) Two-year exposure to 20 ppm CO → 1-1.5% ↑ COHb over control values. c) Two-year exposure to 65.5 ppm CO → 6-8% ↑ COHb over control values.
Smoke	Dini (139)	Man	No statistically significant difference seen in SGPT and SGOT between smokers and non-smokers.
CO (Dosage)	Mazaleski (325)	Rat	a) Cobalt in the nuclear, mitochondrial, and supernatant fraction of liver homogenates was consistently ↑ in rats exposed to 50 ppm CO for 12 weeks. b) Changes in zinc, copper, iron and magnesium were equivocal.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
CO (Dosage)	Ramsey (440)	Man	a) ↑ COHb (mean 5.4%) → ↓ reaction speed to visual stimulation. b) Caused no significant change in depth perception and visual discrimination of brightness.
CO	Goldsmith (180)	Man	a) CO adversely influences performance on: (1) psychomotor tests (2) time discrimination (3) visual acuity. b) 5% COHb sufficient to influence (a). c) Exposures of 50 ppm for 45 min. affected discrimination of the duration of auditory stimuli.
CO (Smoking)	Mantell (320)	Man	↓ Carbonic anhydrase activity in fetal blood seen in infants of smoking mothers.
CO (Smoking)	Rea (443)	Man	No relationship between CO levels and symptoms of respiratory disease or cardiovascular disease, except persistent cough and chest illness could be shown when age was adjusted for.

TABLE 3 (cont.)

BIOLOGICAL RESPONSES OF POSSIBLE USE AS INDICATION OF CIGARETTE SMOKE INTAKE

Component	Reference	Species	Biological Response and Findings
CO (Dosage)	Jones (247)	Rat Guinea Pig Dog Monkey	Chronic exposure to CO (96 ppm) → ↑ hematocrit in all species studied.
Smoke Gas Phase Cambridge + Activated Charcoal Filter Smoke Gas Vapor Phase Cambridge Filter Alone	Driscoll (145)	Rat	a) Freely breathing rats inhaled more in gas phase than in gas-vapor phase as tested by levels of COHb. This was attributed to inhibited breathing caused by the more irritating gas-vapor phase exposure. b) Rats breathing gas phase showed ↑ COHb (19.5 gm%) than rats breathing gas vapor phase (15.5 gm%).
CO (Dosage)	Heistad (209)	Man	a) COHb (19% and 25%) → ↓ reflex vasoconstriction to ice on forehead or lower body negative pressure. b) Inhibition of this reflex may result from COHb → ↑ release of epinephrine.

of the major biological responses cited in the literature is presented in terms of their relevance to use in smoke intake assessment.

1. Nicotine

Nicotine, the principal alkaloid in cigarette smoke, causes many physiological changes in the body. It profoundly influences function of autonomic nervous system giving rise to alterations in gastric secretions, catecholamine production, vasomotor tone, blood pressure, heart rate, etc. Secondarily, changes occur in carbohydrate and fat metabolism, and compensatory responses of the body reflected by changes in other hormones such as insulin, glucagon, and steroids, etc. All these effects are of major physiological importance, and many could be useful in defining smoke intake, but would require close control of those sources which contribute to variability. Without such controls a host of factors that influence the autonomic nervous system would obscure a quantitative relationship between smoking and one of the pharmacological responses to nicotine. Moreover, nicotine is rapidly metabolized and many responses may be terminated within a short time following cessation of smoking. It seems unlikely at this time that the pharmacological responses to nicotine in smoke could be used as a quantitative indication of smoke intake.

2. Immunological Effects

Smoke contains many physiologically active compounds capable of eliciting changes in the immune mechanism of the smoker. Although use of immunological procedures provides sensitive, highly specific end points, it is only within recent times that some of the basic mechanisms have been elucidated. Nilsson[382] showed that smokers had higher levels of serum C-reactive protein (CRP) and that the levels correlated roughly with amount of smoke intake. CRP in blood is a sensitive but non-specific indicator of an active inflammatory or necrotic process. Kosmider[275] reported finding higher levels of immunoglobulin (IgA) and immunoglobulin (IgG) in saliva of smokers. Further research is needed to determine whether these changes in immunological response can be used for determination of smoke intake.

3. Hormonal Response

Smoking has an effect on many hormone levels other than catecholamines. Nicotine is known to be the active agent but other components in smoke may also have an effect. In acute heavy smoking studies in man and with nicotine infusion in rats and dogs, Kershbaum[264] showed a large (> 50%) increase in 11-hydroxycorticosteroids (11-OHCS) in the urine. However, Tucci[529] found no statistically significant difference between smokers and non-smokers in the 24-hour urinary excretion of 11-OHCS, 17-OHCS, or in the serum and urinary levels of cortisol. Moreover, the circadian

variation of cortisol was normal in individuals from both groups. Tucci explains Kerschbaum's finding by saying that Kershbaum's experiments do not accurately represent the true situation. Smokers, he thinks, develop tolerance to the effects of nicotine on the adrenal cortex.

Hendry[210] reported that smokers excrete significantly more anti-diuretic hormone than non-smokers, a finding which is in agreement with those reported by others.[529] No rigorous quantitative relationship between ADH and smoke intake has been reported.

A number of investigators[475,122,134,228,271] have attempted to determine the effect of smoking on serotonin (5-HT) metabolism. The prevailing impression is that smoking probably causes a transient increase in serum 5-HT which is reflected in increased urinary excretion of 5-HT and its principal metabolite 5-hydroxy-indole acetic acid (5-HIAA). However, on a 24-hour basis, 5-HT and 5-HIAA excretion is not significantly different between smokers and non-smokers.[271] Since many other factors influence serotonin, changes in the levels of 5-HT and 5-HIAA are not likely to be a good indication of smoke intake.

Tryptophan, an essential amino acid normally contained in excess in the diet, is metabolized to 5-HT, tryptamine, 3-indole acetic acid, anthranilic acid, cynurenin, N-1-methyl nicotinamide,[71] and possibly cinnabarinic acid.[384] Only tryptamine excretion appears to correlate to some degree to smoke intake during acute episodes of smoke intake.[92]

At this time no hormonal response appears to be specifically or quantitatively related to smoke intake. Obviously, hormone levels are subject to wide individual variation as well as other influences; thus, probably they would not prove to be a good index of smoke intake.

4. Pulmonary Function

Although there are conflicting reports, it is generally accepted that acute smoke exposure does not result in notable alterations in pulmonary function tests.[137,492,128,527,517] For instance, DaSilva[128] reports that smoking one cigarette causes an increase in airway resistance of 11%, while Diamond[137] found that in chronic smokers, airway resistance decreased after one cigarette.

Chronic smoking in some studies,[158] (Camner[531]), but not in others,[26] (Albert[531]), has been reported to depress mucociliary clearance, but there is a large variability among non-smokers as well as smokers. In our experience subject-to-subject variability together with the variability in the methodology rule out mucociliary clearance depression as a means of estimating smoke intake.

In experiments on rats, Lamb[282] and others found that chronic exposure to dilute smoke caused an increase in goblet cell density at all levels of the bronchial tree. Obviously, these changes in the lung are not easily measured without invasion of the body and not sufficiently quantitative to indicate the actual smoke intake.

5. Enzymes

The finding of fluorescent polycyclic hydrocarbons in various body fluids and tissues has been discussed earlier. Of possible interest as a quantitative biological response is the induction of aryl hydrocarbon hydroxylase (AHH) by certain polycyclic hydrocarbons found in smoke.[562,555,259,405,406] The induction of microsomal enzymes of which AHH is one, also alters the rate of metabolism of phenacetin (acetophenetidin).[555,405,406] Conceivably, phenacetin could be administered and its rate of metabolism used to determine the amount of induced enzyme resulting from intake of smoke. Unfortunately, polycyclic hydrocarbons are commonly found in foods, in the environment, etc., and inducibility probably varies with the individual as suggested in experiments by Wiebel[562] and Kellerman.[259] Other drugs such as chlorpromazine are also capable of inducing levels of enzymes which metabolize phenacetin.

Thus, changes in AHH levels or alterations of phenacetin metabolism may not be sufficiently specific for smoking to be a reliable quantitative measure of smoke intake. There is a possibility that other systems could more specifically and quantitatively be altered by smoking and, therefore, be used as a measure of smoke intake.

Chronic elevated carboxyhemoglobin may give rise to biological changes which could indicate something about the duration of the elevated levels of COHb. Astrup[18,19,20] found that elevated COHb levels were associated with an exaggerated shift to the left of the oxyhemoglobin dissociation curve, i.e., more to the left than is predicted by the Haldane equation. The reason for this was a slight reduction in the red cell levels of 2,3-diphosphoglycerate (2,3-DPG)[18] which has been shown to affect the oxydissociation curve. In conditions of hypoxia such as high altitude, Astrup found that 2,3-DPG increased 20-25% during the first 24 hours, and that hemoglobin had a lowered affinity for oxygen as indicated by the rightward shift of the oxydissociation curve. Hypoxia induced by CO paradoxically causes a leftward shift which is further exacerbated by a reduction of 2,3-DPG. Apparently, the change in 2,3-DPG is caused by a minor change in intracellular pH. Levels of 2,3-DPG are also found to be abnormal in patients suffering from acid-base imbalance. Further investigation is necessary to evaluate the possible quantitative information that could be indicated by RBC levels of 2,3-DPG. The changes in 2,3-DPG caused by the CO in smoke must be shown to satisfy the requirements cited previously for a biological marker. Of particular interest are the time constant of the change in 2,3-DPG and the phase relationship of the change in 2,3-DPG to the change in carboxyhemoglobin. If the time constant for the change in 2,3-DPG is longer than the time constant of COHb the level of 2,3-DPG could provide a more integrated value of CO exposure.

E. SELECTION OF POSSIBLE MARKER COMPOUNDS BASED ON ESTIMATES OF AMOUNT OF BODY FLUID NEEDED FOR ANALYSIS

The listing in Table 4 is an attempt to select, of the almost 500 compounds previously cited, those which are worthy of further consideration. The selection, admittedly based on somewhat imprecise criteria, attempts to single out those chemical compounds which have been reported in smoke at levels above those which might be anticipated to be in a reasonable volume of body fluid in terms of sensitivity of existing analytical methodology. The sample size which was used in selecting possible candidates was based on NCI's request that the compound should, if possible, be capable of indicating changes in body fluid from smoking one cigarette. A maximum sample volume of 10 ml was selected because it represents a volume to which most subjects have become accustomed to giving for routine diagnostic tests. Additional compounds can be selected as more sensitive methods become available or if the intake from a greater number of cigarettes is used. In those cases for which different amounts were reported in smoke a range of sample volumes is given.

From the tables it is evident that many of the compounds found in cigarette smoke in larger amounts are ubiquitous, e.g., being found as dietary constituents or in household products. Thus, some thought must be given to possible sources of the marker compound(s) other than from cigarette smoke which can influence levels during intake studies.

The bulk of inhaled water-insoluble, high molecular weight compounds, e.g., paraffins, is probably retained in the lung for relatively long periods. Many of the compounds listed are normal body constituents or are constitutnets found in body fluids in varying amounts in certain disease states. Very short-lived compounds, e.g., acetaldehyde and nicotine, are probably unsuitable as markers for characterizing smoking history since the rate of disappearance requires sampling almost immediately after smoking, and small errors in sampling procedure are likely to give large errors in estimating dosimetry. However, to obtain data to establish the retention by individual smokers following a single puff of cigarette, compounds of this nature may be useful. Estimating daily or weekly intake of smoke would require much more frequent sampling to characterize a continuously varying level. Compounds which are more slowly (or not) metabolized, e.g., water, methanol, and excreted in a manner predictable by appropriate mathematical modeling are better time integrators of smoke intake, since they tend to persist longer in body fluid, e.g., plasma, and with continued smoking to accumulate until a plateau is reached. With the necessary pharmacokinetic data, short-term smoke intake, e.g., daily intake, can be determined by noting the level at specific time periods during the period of increasing concentration which occurs within a fixed time after smoking. The plateau in concentration, when achieved, will also provide data on smoke intake. In summary, complete pharmacokinetic data are needed to assess the relative merits of possible markers of smoke intake. In its absence, all degrees of speculation are possible.

TABLE 4

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Alcohols		
Furfuryl alcohol	1.1	Found normally in some foods.
Glycerol	0.15	Common metabolic product; a physiologically abundant compound. Found in dietary sources.
Methanol	7-3.1	Associated with alcoholic beverages. Food contaminant. Also in coffee, vegetables, cocoa, tea, fruits, citrus and essential oils. Also found in some household products.
Menthol	3.1-1.1	Candies as a source. Also flavor for some food. Varying amounts added to some brands of cigarettes--level varies with storage.
Propylene Glycol	1.9-0.6	Medication solvent; food solvent.
Aldehydes		
Acetaldehyde	1.1-0.7	Product of ethanol oxidation by alcohol dehydrogenase and product of pyruvate decarboxylase. Commonly found as environmental contaminant. Found in fresh water (0.2 mg/liter).*
Acrolein	11.7-7.6	Metabolic product of propionyl CoA. Commonly found as environmental contaminant. Found in cooked fats, coffee, cocoa, tea and vegetables. Found in fresh water (0.01 mg/liter).**

*Ref. #15 *in:* Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

**Ref. #136

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Ketones		
Acetone	1.8-0.011	May be found in body fluids, especially during certain metabolic diseases. Can occur as product of fatty acid degradation. Found in beer and wine.
2-Butanone	7.9	Paint solvents, adhesives, etc.; coffee and in some flavorings. Found in fresh water (1.0 mg/liter).*
Maltol (2-hydroxy-3-methyl-4-pyrone)	1.3-0.6	Needs further study. Flavoring in Kool-aid, etc. Found in burnt sugar, flour.
Esters		
Triacetin (glyceryl acetate)	3.2-2.1	Triacylglycerols are common physiologically. Possibly in milk. In cigarette filters.
Formate ester	0.17	In flavorings, coffee, cocoa, tea, citrus oils, fruits.
Ethers		
Guaiacol (o-methoxyphenol)	0.48-0.25	Needs further study. Occasionally used as medicinal. In celery seed and coffee. Occasional flavoring.

*Ref. #227,422 *in:* Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Aliphatic Hydrocarbons		
n-Butane	11-9	May be applicable but frequently present in household products, e.g., cigarette lighters. Gasoline vapors.
2-Methyl-1,3-butadiene	1.7-0.01	Gasoline vapors.
2,3,-Dimethyl-1-butene	10	Gasoline vapors.
2-Methyl-2-butene	12-9	Gasoline vapors.
Ethane	2.1-1.3	Gasoline vapors. May be applicable.
Ethylene	3.6-2.6	May be applicable. Found in ripening fruit. Found in fresh water (0.2 mg/liter).*
1-Hexene	1.96	
trans-2-Hexene	7.7	
Limonene (Dipentene)	5-3	Present in fruits, candies, citrus oils, skin of lemons.
nC_{30} alkane	53-6.3	Ubiquitous in surface lipids of living organisms. Poorly absorbed from lung.
nC_{31} alkane	32-1.8	
nC_{32} alkane	32-3.2	
nC_{33} alkane	105-2.5	

*Ref. #355 *in:* Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Aliphatic Hydrocarbons (cont.)		
Methane	0.8-0.005	Needs further study. Found in bowel. Bacterial action in gut. Frequently present in household heating gas. Found in well water (0.8-87 ml/liter).*
Cyclopentane	6	
2-Methyl-2-pentene	2.6	
4-Methyl-2-pentene	1.9	
Propane	3.5-2.5	May be applicable. Frequently found in household products.
Propene	3.2-2.6	
2-Methylpropene	9.8	
Aromatic Hydrocarbons		
Toluene	14-7.9	May be applicable. It is a common industrial solvent. Found in paint solvents, brush cleaners, adhesives. Found in fresh water (1.0 mg/liter).**

*Ref. #291 *in:* Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

**Ref. #2

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Phenols		
Phenol	0.08-0.03	Polyphenol found in fruit trees. Possibly in common disinfectants, cleaning solutions. Used as antiseptic, medicinal. Found in fresh water (0.001 mg/liter).*
m- + p-Cresol	15-10	Possibly in common disinfectants, cleaning solutions. In fermented beverages.
Nitriles		
Acetonitrile	5.5-0.0063	Found in milk and cheese. Has been studied. Higher levels in smokers, but not related to number of cigarettes.
Hydrogen Cyanide	1.6-0.02	Has been studied especially for smoke intake (as thiocyanate). Found in some foods. Amygdalin of almonds yield HCN upon hydrolysis.
Sulfur Containing Compounds		
Hydrogen Sulfide	7	May be produced by bacteria in gut. Putrifaction. Found in environment; eggs.

*Ref. #103, 227 *in:* Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Amines		
Isoamylamine	1.96	Found in apples.
Aniline	3.1	Product of enzymic decarboxylation of anthranilic acid. Found in fresh water (0.1 mg/liter).*
n-Butylamine	2.6	Microbial decarboxylase produces this from norvaline; probably not physiologically significant.
Isobutylamine	2.6	
Ethylamine	0.8	Found in fish. Product of alanine decarboxylation, although not sure whether such an enzyme has been found. Found in fresh water (0.5 mg/liter).**
Methylethylamine	4	Found in fish. Found in fresh water (1.0 mg/liter).***
β-Phenylethylamine	0.76	Found in fish. Product of decarboxylation of phenylamine.
Methyl-β-phenylethylamine	5.3	
Methylamine	0.4	Product of decarboxylation of glycine, but do not know of a glycine decarboxylase.
Dimethylamine	0.8	Found in fresh water (0.1 mg/liter).****
n-Propylamine	4	Product of decarboxylation of α-aminobutyric acid (microorganism enzyme).

* Ref. #217,455
** Ref. #117
*** Ref. #328
**** Ref. #88,89

in: Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Carboxylic Acids		
Acetic Acid	5.4-0.6	Physiologically abundant compound (PAC). Found in dietary sources (in fruits). Found in home products.
Formic Acid	6.7-0.0013	By-product of methanol intake. In ants.
Furoic Acid	21-4.5	
Glycolic Acid	12.6-2.1	Accumulates in glycolic aciduria.
Lactic Acid	12.6-2.1	PAC; and found in dietary sources.
Linoleic Acid	0.13-0.04	PAC; and found in dietary sources.
Linolenic Acid	0.04-0.02	PAC; and found in dietary sources.
Levulinic Acid	32-9	PAC; and found in dietary sources.
Malonic Acid	7	PAC; and found in dietary sources.
Oleic Acid	0.3-0.06	PAC; and found in dietary sources.
Oxalic Acid	16-7	PAC; and found in dietary sources.
Palmitic Acid	0.04-0.02	PAC; and found in dietary sources.
Propionic Acid	16-2.1	PAC; and found in dietary sources.
Succinic Acid	6.3-2.5	PAC; and found in dietary sources.
Stearic Acid	0.2-0.07	PAC; and found in dietary sources.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Inorganics		
Argon	0.001	Universal distribution.
Hydrogen	1.8-0.009	Universal distribution.
Nitrogen	3.7×10^{-5} - 2.8×10^{-5}	Universal distribution.
Oxygen	1.4×10^{-2} - 1.1×10^{-4}	Universal distribution.
Water	0.001	May be applicable if tagged, despite PAC; and found in dietary sources. Universal distribution.
Cadmium	2.9-1.1	Frequently found as dietary component. Has been found in tissues and fluids.
Chromium	27-6.7	Has been found in tissues and fluids.
Copper	3.3-1.4	Known to be an important nutrient factor. PAC; and found in dietary sources.
Lead	7-3	Has been found in tissues and fluids, accumulates in metallothienien.
Nickel	6-1.7	Found in tissues.
Zinc	0.9-0.06	PAC; and found in dietary sources. Essential role in metabolism as enzyme cofactor.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Heterocyclic N		
Carbazole (Dibenzopyrrole)	9-6.29	
Dimethylindole	6.3	
Piperidine	0.9	
Δ^3-Piperidine	8.3	
Pyridine	21-4.5	Found in fresh water (0.2 mg/liter).*
3-Methylaminopyridine	1.02	
Nicotine	0.002-0.001	Older types of insecticides "Blackleaf 40".
Pyrocoll	6.25	
Cotinine	10.5	
Pyrrolidine	0.26	

*Ref. #227,464 *in:* Water Quality Criteria Data Book, Vol. I: Organic Chemical Pollution of Fresh Water, Environmental Protection Agency, Water Quality Office, December, 1970.

TABLE 4 (cont.)

SELECTED COMPONENTS IN CIGARETTE SMOKE OF POSSIBLE USE FOR ASSESSMENT OF SMOKE INTAKE

Component	Sample Size per Cig. (ml or gm)	Applicability
Miscellaneous		
Acetamide	0.17-0.11	Given off by mice.
Carbon Dioxide	0.02-0.0009	May be applicable. Abundant in tissues. Found in high levels in some environments. Found in beverages, rapid disappearance.
Carbon Monoxide	4.6-0.004	May be applicable. Frequent atmospheric contaminant. Small amount produced endogenously.
Formamide	1.59-2.3	
Methyl Chloride	3.3-0.007	
Methylisocyanate	1.3	
Methyl Nitrite	40-1.4	
Propionamide	1.05-0.25	

F. SELECTED BIOLOGICAL RESPONSES AS POSSIBLE INDICATORS OF SMOKE INTAKE

The number of biological responses cited in the published literature dealing with nicotine alone number into the thousands. Of these only a few are applicable to this study since nicotine was used as a tool to study the pharmacology of drugs or agent unrelated to cigarette smoke. Where biological responses after smoking or after dosing with a compound found in smoke are of potential value in estimating smoke intake, the citation was discussed in relationship to similar studies by other workers. Such responses as changes in heart rate, blood pressure, coronary blood flow, hematocrit, visual discrimination, electrocardiogram, platelet aggregation, various diseases, e.g., atherosclerosis, are likely to be poor choices since these responses are easily evoked by a multiplicity of emotional and environmental factors. Some of the problem areas we anticipated would be associated with use of these biological responses are also included.

1. Effect of Nicotine on Catecholamine Levels in Blood and Urine

a. Nicotine (smoke) is followed by elevation of blood catecholamines.[269,262,261]

b. (1) Urinary norepinephrine (NE) is significantly increased in smokers compared to non-smokers.[206]

(2) Urinary NE is not significantly increased in smokers compared to non-smokers.[560,529]

c. (1) Urinary epinephrine (E) is significantly increased in smokers compared to non-smokers.[560,160]

(2) Urinary E is not significantly increased in smokers.[529]

Comment

Depending on dose, nicotine has been shown to stimulate as well as block various autonomic functions. An increase in epinephrine and norepinephrine in the blood has been reported. This increase should be reflected in increasing urinary excretion of both norepinephrine and epinephrine or their metabolites. However, only about 5-10% of total catecholamines remains unchanged in the urine. Many factors affect catecholamine levels in serum or urine which are not directly related to smoke intake.

2. Lipid Metabolism

a. Smoking is followed by an increase in non-esterified fatty acids.[269,99]

b. Smoking is followed by an increase in free fatty acids.[99,144,299,341,262,261,100]

c. Smoking one cigarette is followed by a decrease in the level of blood cholesterol.[194]

d. Smokers of greater than 10 cigarettes per day have higher levels of blood cholesterol than non-smokers.[427,80,447,566,99,341,64]

e. Recent studies indicate that the association between smoking and serum cholesterol is slight to non-existent; smoking is an independent risk factor for atherosclerotic disease (i.e., shows a positive association independently of serum cholesterol levels).[588,589]

f. Smoking one cigarette leads to a decrease in blood levels of β-lipoproteins.[194]

g. Smokers of greater than 10 cigarettes per day have higher levels of β-lipoproteins.[194,80,64]

h. Smoking 4 cigarettes leads to an increase in the rate of *in vitro* cholesterol crystallization.[265]

i. Smoking leads to an increase in the level of blood triglycerides.

Comment

Epinephrine has been shown to increase plasma lipids. Since nicotine stimulates the release of epinephrine from the adrenal medulla and norepinephrine from other adrenergic nerve endings, free fatty acid levels in blood would be expected to be increased. However, many other factors influence fat mobilization, e.g., diet, insulin, glucose, corticosteroids, and thyroid hormone. Stress and time of last meal also influence blood levels. Thus, levels of fatty acids and other fat constituents in plasma would not be a good indicator of smoking habits.

3. Hormones

a. Smoking is followed by a transient increase in serum serotonin (5-hydroxytryptamine; 5-HT).[122]

b. Blood levels of 5-HT are comparable between smokers and non-smokers prior to smoking.[122]

c. (1) Urinary excretion of 5-hydroxy-3-indole acetic acid (5-HIAA)/24 hours is higher, but not significantly, in smokers.[271,483,484,475]

(2) Urinary excretion of 5-HIAA is not increased in smokers[134,228] or after smoking.[134,228]

(3) Non-smokers excrete significantly more 5-HIAA after smoking.[475]

d. Smokers show no significantly different excretion of tryptophan or niacin metabolites such as cynurenin, anthranilic acid, and N-1-methyl nicotinamide.[71,4]

e. There is an approximately linear increase in urinary elimination of tryptamine/24 hours compared with the number of cigarettes smoked.[92]

f. Histamine: Smoking was followed by an increase in the excretion of methyl imidazole acetic acid, a metabolite of histamine.[183]

g. Steroids:

(1) Smokers did not have significantly different urinary excretion of 11-hydroxycorticosteroids (11-OHCS), 17-OHCS, or cortisol. Circadian rhythm of cortisol in serum was normal in smokers.[529]

(2) Acute smoking (4 cigarettes/30 minutes) is followed by an increase in the excretion of 11-OHCS.[264]

Comment

Smoking appears to influence certain pathways associated with tryptophan metabolism. At present, there does not seem to be a clear relationship between smoking and levels of serotonin (5-HT) and its metabolites, or between smoking and levels of metabolites in other pathways related to tryptophan metabolism. Additionally, there is no clear evidence that chronic smoking affects urinary excretion of 11-hydroxycorticosteroids. So many variables influence these biochemical pathways that a change in a metabolite probably would not reliably indicate a change in smoking habits.

4. Vitamin Levels in the Body

Ascorbic Acid (Vitamin C):

a. Smokers have significantly less Vitamin C (ascorbate) in their plasma than non-smokers.[411,412,67]

b. Smokers have significantly less ascorbate in their leukocytes than non-smokers.[67]

c. At low doses of 58-98 mg/day of ascorbate, smokers excrete 40% less ascorbate than non-smokers.[411,412]

Vitamin B_{12}:

a. Smokers have decreased levels of serum B_{12} than non-smokers and also excrete more B_{12} in the urine.[306]

b. Smokers excrete more SCN^- in urine than do non-smokers.[306,575,135,285,33,141,574]

c. Smokers have higher levels of SCN^- in blood than non-smokers.[165,48,37,415,135,61,416]

Comment

Vitamin B_{12} and Vitamin C levels may be influenced by smoking. In smokers plasma and leukocyte Vitamin C are reduced. Since smokers excrete less of a loading dose of Vitamin C, both reduced absorption by smokers of the ingested amounts and altered utilization of Vitamin C are possible explanations. Vitamin B_{12} is related in several ways to detoxification of ingested and inhaled CN^-. The variability among individuals of their dietary intake of Vitamins C and B_{12} would certainly contribute to the poor correlation between smoking habits and serum and urinary levels of these vitamins.

5. Induction of Enzymes

a. Plasma phenacetin levels following a 900 mg oral dose were significantly lower in smokers than in non-smokers, and plasma levels of the principal metabolite of phenacetin, N-acetyl-*p*-aminophenol (APAP) were also decreased in smokers, but not significantly.[405,406]

b. (1) In rats previously dosed with 3,4-benzpyrene (3,4-BP), the concentration of phenacetin following I.P. injection was decreased more significantly in the whole body homogenates than in controls.[405,406]

(2) An *in vitro* culture of intestinal mucosa from rats previously dosed with 3,4-BP transformed phenacetin to APAP at a higher rate than cultures from control rats.[405,406]

c. (1) Smoking causes the induction of aryl hydrocarbon hydroxylase (AHH or benzpyrene hydroxylase) in rats.[555]

(2) Degree of tissue inducibility varies from tissue to tissue.[555,562]

(3) AHH can be induced in mice by injection or topical application of benz(a)anthracene.[562]

d. Sputum from smokers has higher incidence of fluorescent histiocytes and the fluorescence is more intense than in non-smokers. There appears to be rough correlation between intensity and smoke intake (Vassar[536]).

Comment

Experiments in animals and man indicate that smoking results in an increase in the intake of polycyclic hydrocarbons and leads to the production of an inducible enzyme, aryl hydrocarbon hydroxylase (AHH, also benzpyrene hydroxylase). Increased enzyme activity results in higher rates of metabolism of phenacetin. Apparently phenacetin itself does not cause induction of the microsomal enzymes responsible for its own metabolism.

It is unlikely that the induction of these enzyme systems could be used as an index of smoke intake since as shown in rats of different strains, considerable variability exists among individuals. Also other drugs have been shown to alter the rate of metabolism of phenacetin (e.g., chlorpromazine).

The finding of several papers of fluorescence in tissues (or body fluids) after smoking is an interesting approach and could prove of value as an index of smoke intake. It is likely that the fluorescence is due to the polycyclic hydrocarbons which are found predominantly in the particulate fraction.

6. Miscellaneous Responses to Smoking

a. There is a small and statistically insignificant increase in the partial pressure of nitrogen (pN_2) in blood and urine of smokers compared to non-smokers. This might suggest the presence of an increased ventilation perfusion inequality in smokers.[396]

b. Serum of smokers compared to that of non-smokers has an increased precipitating titre of immunoglobulins IgM and IgA and decreased titre of immunoglobulin IgG and isoagglutinin anti-A. The saliva of smokers has an increased precipitating titre of immunoglobulin IgA and IgG.[275]

c. One aspect of the toxicity of nitrogen dioxide (NO_2) is the effect on hemoglobin since NO_2 probably interacts with hemoglobin forming two types of non-heme iron complexes.[459,460] The question arises, will analysis of blood for the amount of NO_2 bound to hemoglobin give a quantitative and reliable estimate of amounts for the amount of concentration of NO_2 in the smoke?

IV. BIOCHEMICAL CONSIDERATIONS RELATED TO PROBLEMS OF SELECTING METHODS SUITABLE FOR QUANTITATIVE ASSESSMENT OF SMOKE INTAKE

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Clearly, one of the most arduous tasks in selecting an appropriate method for assessing smoke intake is screening the great bulk of published data on biological and chemical aspects of cigarette smoke for a very limited number of studies relating to methods to monitor smoke intake. Some requirements and the major problem areas are discussed briefly below.

A. PROPERTIES OF MARKER COMPOUND

A component suitable for use as a marker to monitor smoke intake should not show a marked difference in concentration as the cigarette is smoked to a shorter butt. The transfer of a suitable smoke marker compound to body tissue requires that the marker not be greatly influenced by condensates which distill through the cigarette near the butt end. Some degree of compromise will be necessary, as indicated in Fig. 1. The data based on a non-filter cigarette (filter cigarette would give different curves) show that amount of condensate, water and alkaloids in the last few puffs is considerably higher than that of the first 6 puffs. This is less of a problem with carbon monoxide. It is also evident that similar results for smoke intake might be expected with condensates, alkaloids (notably nicotine) or a gas (carbon monoxide), if only 6 puffs are inhaled. With 7, 8 or 9 puffs, carbon monoxide would give perhaps a better indication of smoke intake than if particulates or water is used.

To eliminate the problem of differences in tobacco weight, tar yields, nicotine, etc., one approach is to base cigarette consumption on the number of cigarettes smoked. The concentration of the marker compound would not vary appreciably between cigarettes of the same brand or different brands. Moreover, the smoke from different cigarettes should have approximately the same level of marker. We recognize, however, there will be differences in tobacco additives, weight of tobacco, draw resistance, humidity, temperature, etc., all of which must be considered and accounted for in interpretation of the data. Some adjustments can be made for differences in smoke yields and components by comparing analytical data with the amount of marker in smoke.

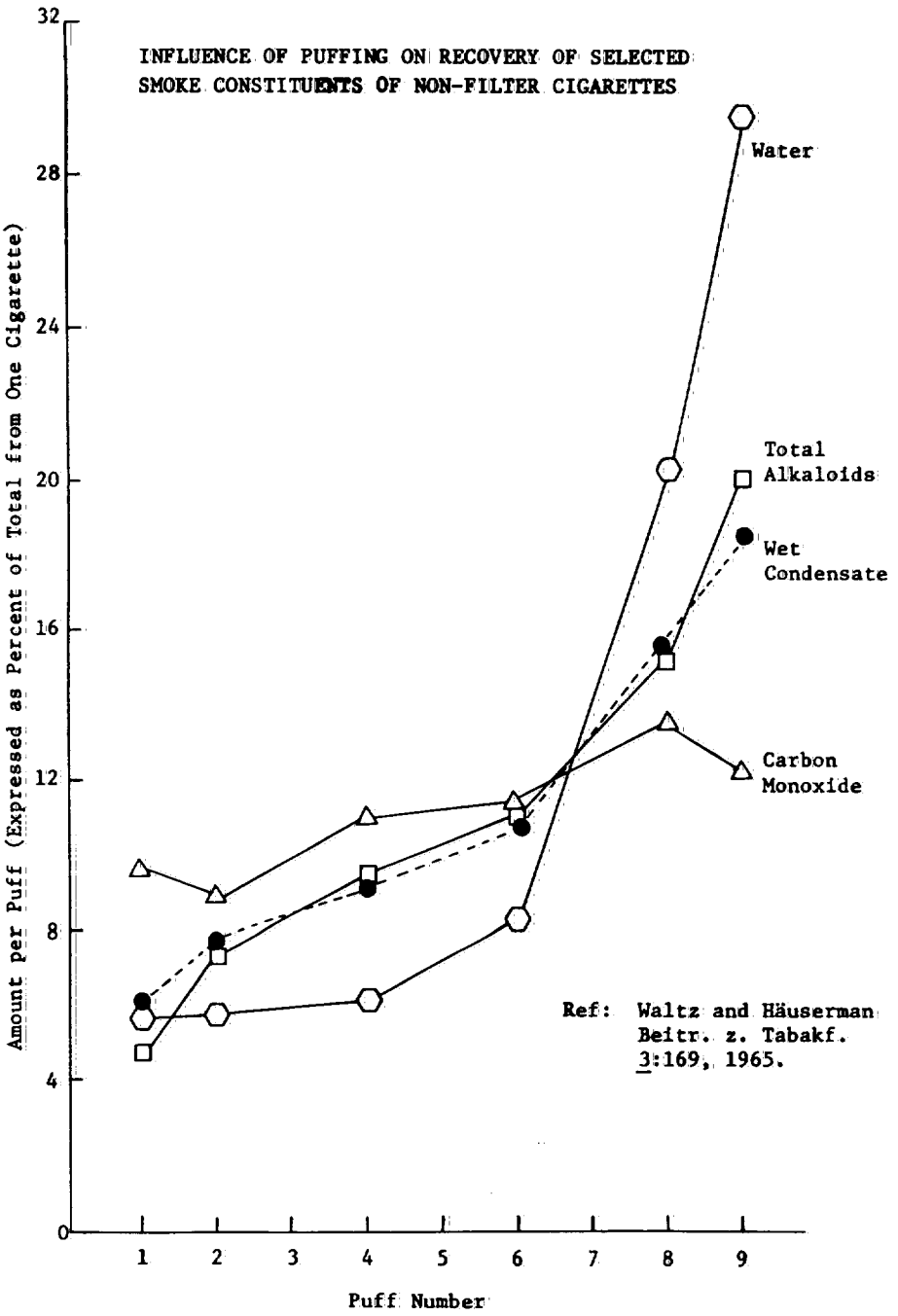


Fig. 1

B. BIOLOGICAL FATE OF MARKER COMPOUND

The marker component should not deposit in the respiratory tract in a non-diffusible depot, but should move freely across mucosal membrane and distribute uniformly in the appropriate body fluid. It should not undergo extensive metabolism or induce metabolic changes unless the metabolites or changes are known and quantitative.

Because smoke is made up of small size particles, most workers have assumed the transfer of particulate components to be almost instantaneous. Lindquist and Ulberg[586] have shown that mice given a single dose (I.V.) of C^{14} nicotine retain high concentrations of label in the respiratory tract for up to 30 days. If a significant amount of binding occurs, it could greatly influence transfer of marker compounds to body fluids, and thus profoundly affect the validity and outcome of smoke intake studies. Thus, we recommend that the rate of uptake from the lung to an appropriate body fluid, e.g., plasma, be determined for any compound selected as a marker for estimating intake of smoke particulates.

C. RETENTION OF MARKER COMPOUND

The compound of mainstream smoke should not have a very rapid elimination rate. It should persist in body fluids long enough to provide adequate levels and time for sampling and analysis, but not so long that the body fluid does not reflect a major change in smoke intake.

D. MEASUREMENT OF MARKER COMPOUND IN BODY FLUID

A suitable marker compound must not be one normally found in the body fluid at high concentrations relative to its concentration in smoke, nor should it be available in body fluids from environmental or dietary sources. It should come only from cigarette mainstream smoke. The level in the body fluid should reflect only the amount of smoke inhaled and thus be independent of individual modes of smoking, e.g., mouth puffing vs. inhalation, frequent shallow vs. occasional deep inhalation. Unfortunately many of the major components in smoke are available from endogenous and dietary sources necessitating adequate controls.

E. ANALYSIS

Analytical methodology should not require costly, time-consuming, elaborate isolation and identification procedures to determine the amount of the marker compound in body fluid. It should be simple, rapid, specific and reproducible in order to provide reliable yet inexpensive estimates of smoke intake. Costs for reproducible, high sensitivity, rapidly responding analytical instruments may require high capital outlays initially, but be more economical in the overall program costs.

F. GASEOUS VS. CONDENSATE MARKERS

The decision of whether the marker compound should be a component of the gas or particulate phase is very important since the localization and distribution between particulate and gaseous components may differ widely. In human smoking, inhalation occurs through the mouth. Thus, selection of a gaseous marker compound would probably be a reasonable means of estimating smoke intake provided the bulk of the compound was not absorbed, e.g., water soluble gases, by the larger airways, rapidly cleared or absorbed by the saliva in the mouth and also swallowed.

Use of a particulate marker has the advantage in that it represents the phase of greatest biomedical interest and is more representative of actual "smoke" retention. Gases have the advantage that the molecules distribute throughout the lung in a more uniform fashion, are capable of more rapid diffusion into systemic circulation, and distribution into body fluids. Particles, on the other hand, are deposited non-uniformly according to size, mass, velocity, impaction, etc., and may collect in certain discrete areas. Components of very small particles that reach the fine pulmonary structure will rapidly diffuse across the epithelial lining and become absorbed into the pulmonary circulation, whereas larger particles will be removed in larger airways and absorption of their components will probably be very slow unless cleared from the trachea, swallowed and abosrbed by the gastrointestinal tract.

Thus, two markers might be needed to reliably measure smoke retention; a gaseous compound and a predominantly particulate compound. A single marker known to distribute in both phases in a relatively predictable manner might also be acceptable.

G. BODY FLUIDS

For the purposes of this study a liberal interpretation has been given to the term "body fluid" in not restricting it to the usual connotation of a "fluid" being a liquid. Before a critical evaluation of suitable marker compounds and analytical methodology can be made, consideration was given to the different body fluids available for sampling and the merits of each.

Blood and urine are the two body fluids that have been most commonly used for clinically characterizing a subject's physiological and biochemical status. Other fluids include body surface effluents, saliva/sputum, lacrimal fluid (tears), lymph, bile, and finally, expired air. The following are salient features of each of these body fluids as related to the problem of measuring marker agent(s) as indicators of smoke intake. Lymph, the fluid that fills the extra-vascular spaces, and bile, the fluid produced by the liver, can be eliminated from further consideration due to their inaccessibility for sampling purposes.

1. Blood

This body fluid makes up 5 liters of every normal adult 70 kg male, somewhat less of the female. Approximately 3.2 liters of this volume is plasma and 1.8 liter is formed of cellular elements. Blood participates in virtually every functional activity of the organism and maintains a constant cellular environment by circulating through every tissue; delivering nutrients to the tissue and removing metabolic waste products for excretion. The chemical composition of blood is extremely complex and contains many of the same chemical or related compounds found in smoke.

The principal advantages in sampling blood (or plasma or serum) are: (a) the relatively short time in which agents absorbed by the lung appear in increased concentration in blood, (b) once absorbed, there is rapid mixing of agents with the entire blood volume. This provides, for most compounds, a more representative sample for characterizing total body burden. The sample volume requirements determine in part the degree of invasiveness needed to obtain the necessary amount of blood. Analytical methods requiring 0.1 ml of blood can be obtained by pinprick of a finger, whereas larger volumes, e.g., 5-10 ml, require more invasive measures, e.g., venipuncture. An analysis of blood in general requires separatory procedures to remove interfering materials and/or concentrate the compound. Blood has been frequently used in past efforts to estimate smoke intake and usually has involved nicotine, carbon monoxide, or thiocyanate analysis.

2. Urine

This body fluid, formed by the kidney, is a complex solution of organic and inorganic substances resulting principally from metabolism. The composition of urine varies greatly depending upon food and water intake, metabolic by-products, disease, etc. Since the kidney is also the major excretory organ, most of the foreign chemicals, e.g., drugs, which find their way into the body are excreted in urine. Thus, the bulk of the agents in cigarette smoke which are inhaled may be expected to be excreted in urine either unchanged or as metabolites.

Urine as a body fluid is capable of reflecting total body burden for a great variety of chemical compounds. In some cases, e.g., those which have low renal thresholds and distribute in body water, the concentration in urine is a reasonably reliable means of estimating the body burden. For agents which are reabsorbed by the kidney or absorbed into body depots, urine is less reliable. The advantages of sampling urine are: (a) urine collection is completely non-invasive and sampling requires only the voiding into an appropriate container, (b) the volume of the sample is large (typically greater than 100 ml), (c) chemical analysis is easier than with blood due to the almost complete absence of cellular elements and the low concentration of protein and lipids. In addition to poor correlations between levels in blood and urine following a fixed body burden rapid changes are difficult to measure since urine is accumulated in the bladder between voidings. This results in discontinuous sampling which averages over time the changes in concentration between voidings. Urine has been used to estimate smoke intake principally for analysis for nicotine (cotinine), thiocyanate, acetonitrile, and polonium-210.

H. BODY SURFACE EFFLUENTS

These body fluids include perspiration, a fluid produced by the glands located in the skin and also includes chemicals that diffuse through the skin. Obtaining samples of perspiration for chemical analysis can be a problem from the standpoint of quantitative collection. The rate of perspiration is extremely variable and depends upon the ambient temperature (almost zero at lower ambient temperature), humidity, and the amount of physical activity.

Analysis of perspiration, together with agents which are excreted by sebacious glands (also found in the skin) or diffuse directly through the skin, have been used to measure body burden of a variety of compounds. Samples may be obtained by rinsing the skin with known volumes of water or other suitable vehicles, by absorbing the fluid on a suitable medium, e.g., filter paper, or collecting effluents by flushing the body surface from all or a known segment of the body with a pure inert gas. Analyses are then carried out to estimate the rate of excretion. Ordinarily the precision possible with body surface effluents is likely to be much lower than with other body fluids.

With the increased sensitivity available from recent advances in gas chromatography and mass spectrometry analyses of agents in body surface effluents will undoubtedly be more extensively studied. For example, Drs. Dravniek and Krotoszynski[585] have used gas chromatography to characterize odors picked up in highly purified air passed over the skin of human subjects enclosed in special glass tubes.

The use of body surface effluents as an effective means of measuring smoke intake depends upon the degree of correlation between amount of smoke inhaled, the concentration in the blood, the rate of elimination from the skin as well as the amount of time, and the degree of personal discomfort involved in sampling.

Components of smoke such as carbon monoxide and many other gases, nicotine (or cotinine), acetonitrile, cyanide (or thiocyanate), may be expected in body surface effluents in relatively small, but possibly in measurable amounts.

1. Saliva and Sputum

Saliva is a fluid formed in various exocrine glands of the oral cavity. About 1 to 1.5 liters per day are secreted in response to either stimulation of local sensory nerve endings or from impulses arising from centers in the CNS. Sputum is the fluid coughed up from the bronchopulmonary tree. Unless removed from the trachea directly,

samples of sputum also contain saliva. Both saliva and sputum have been collected for study of effects of cigarette smoke on various pulmonary structures, e.g., histiocytes, macrophages, surfactant, enzymes, etc. Saliva has also been used to determine smoke intake by analysis of thiocyanate, nicotine and metabolites of nicotine.[141] Unlike sputum which is relatively inaccessible, samples of saliva are easily obtained in reasonably large volumes.

2. Lacrimal Fluid

Lacrimal fluid (tears) is secreted by glands of the eye. Ordinarily the rate of drainage is sufficient to prevent accumulation of fluid in the eye. Although secretory activity can be increased, e.g., through local irritation or emotional factors, collection of adequate amounts of lacrimal fluid for analysis to determine smoke intake may present a problem and probably represents a poor choice for smoke intake.

I. RESPIRATORY GASES

Many compounds are known to be excreted in the breath, and included are the well known volatile anesthetics and alcohol. For a compound to be a useful marker for smoke intake, a high degree of correlation between blood levels and levels in the expired air must exist. Rea et al.,[443] Cohen et al.,[107] and others have shown a reasonable correlation between expired CO in breath and CO saturation of blood. The advantages in using respiratory gases are: (1) that samples are easy to obtain, i.e., methods are rapid, completely non-invasive, and (2) in sufficient concentrations gases are usually the easiest fluid to analyze. For these reasons many of the previous attempts to estimate smoke intake have used breath analysis to measure body load.

In the experience of law enforcement officials with alcohol breath analysis, the correlation between blood and breath alcohol is reported to be adequate so that in some states it is used to legally define the level for "drunk-driving." In this setting, however, the levels of alcohol in the breath for "drunk-driving" are much higher than levels of components encountered in the breath of smokers. With few exceptions most of the research using breath to measure smoke intake has involved carbon monoxide, since it is found in smoke in rather high concentrations and is excreted virtually unchanged via the lung. Carbon monoxide in expired breath has been shown by Rea et al.,[443] and Stewart[511] to generally correlate with the number of cigarettes smoked. Rea has also shown that carboxyhemoglobin saturation correlated better with individuals who inhaled, and less correlation if they attempted to quantitate the carboxyhemoglobin with the depth of inhalation during smoking.

In addition to carbon monoxide, hydrogen, methane, and methanol may provide alternative markers to assess smoke intake, but additional study would be required to determine endogenous and exogenous background levels from levels due to smoking.

V. SELECTED BIOANALYTICAL METHODOLOGIES AND ANALYTICAL INSTRUMENTATION APPLICABLE TO SMOKE INTAKE ANALYSIS

V. SELECTED BIOANALYTICAL METHODOLOGIES AND ANALYTICAL INSTRUMENTATION APPLICABLE TO SMOKE INTAKE ANALYSIS

A. INTRODUCTION

It is the purpose of this section to discuss briefly recent applications of analytical techniques and describe current developments and/or modifications in scientific instrumentation that would provide greater sensitivity and specificity for possible use in quantitation of components for estimating smoke intake. Developments in five pertinent areas will be considered: mass spectrometry, gas-liquid chromatography, radioimmunoassay, stable isotopes, and spectroscopy.

B. MASS SPECTROMETRY AND RELATED TECHNIQUES

Mass spectrometry is probably the most comprehensive and versatile of all instrumental methods of analysis. Almost any material can be analyzed with a suitable mass spectrometer, and more information is obtained per microgram of sample than with any other analytical technique. However, this instrument has its limitations; e.g., it cannot identify stereoisomers. When used in conjunction with nuclear magnetic resonance, ultraviolet and infrared spectroscopy, mass spectrometry becomes even more useful in accurately solving complex structural problems. The use of computers in acquiring and processing mass spectral data is becoming more widespread and in some laboratories small computers are now considered an integral part of the instrument. The computer can also be used to program the mass spectrometer, in addition to data processing.

Although mass spectrometers have been used extensively in biochemistry for over 15 years, recent adjuncts to the basic instrument (different techniques in molecular ionization, and the combined use of stable isotopes with mass spectral analysis) has added new dimensions to this important method of analysis. Moreover, the mass spectrometer along with varying techniques of operation has continued to fill the ever-pressing needs of sensitivity and specificity, and these developments are discussed below.

1. Electron-Impact Mass Spectrometry (EI)

Of all the methods which have been used to ionize atoms and molecules, electron impact is by far the most frequently employed. This is partly due to the fact that intense electron beams of sufficient energy can be produced with relative ease and, furthermore, that the energy of the electron can be varied in the desired range in simple ways. The pressures of the ionization chamber are usually lower than 10^{-4} torr, and because of this, only primary ionization processes are usually observed.

The ionized molecule usually has received sufficient energy to result in fragmentation of some of the chemical bonds. The resulting assemblage of positively charged molecular and fragment ions can now be controlled by the "electrostatic optics" of the ion source. The ions are focused into a well-defined ion beam and accelerated out of the source by a series of negatively charged (with respect to the ionization chamber) plates. All the ions receive the same potential energy (typically 2-3 KV) in the acceleration section and thus have the same kinetic energy ($1/2\ mv^2$). However, since the ions have different masses, they must have different velocities and upon entering the magnetic field, their paths follow different radii of curvature. Thus, the magnetic field disperses the total ion beam into a spectrum of various ions according to mass/change ratio (m/e). Most instruments are operated with a varying magnetic field and thus the entire mass spectrum can be drawn across the detector slits by scanning either the magnetic field strength or the accelerating voltage.

Positive ions may be detected electrically or photographically; the technique usually depends on the application.

2. Ion-Molecule Reactions - Chemical Ionizations Mass Spectrometry (CI)

The chemical reactions of ions in binary collisions with neutral molecules (reactions which are extremely rapid compared with ordinary chemical reactions) are defined as ion-molecule reactions.[700] Unlike other reactions taking place in the ion's source, which are unimolecular, ion-molecule reactions are bimolecular and are easily recognized because their occurrence is proportional to the square of the sample pressure. The collision of ions with neutral molecules is relatively rare under the low-pressure conditions, less than 10^{-5} torr, which usually prevail while the mass spectra of organic compounds are measured. In contrast, gas phase ion-molecule reaction studies are carried out of relatively high ion source pressure.

Ion production by chemical ionization consists of reacting the sample being investigated with a known and preselected set of ionizing reactant ions.[701-704] To accomplish this the ion source is operated at relatively high pressure ($\sim$ 1 torr) by the addition of a reactant gas which is ionized by a combination of electron impact and ion-molecule reactions which ultimately lead to ionization of the sample molecules bled into the ion source. The significant differences between EI and CI are related to three factors: CI is not governed by Franck-Condon considerations but involves slow equilibrium adjustments of electronic states and atomic positions; CI produces, initially, ions with an even number of electrons; and lastly, although most importantly, the amount of energy transferred to the initial product ions is low, being highly dependent on the reactant gas in use.

In general, CI mass spectra show few ions and more intense high-mass ions than do EI mass spectra, a factor of considerable importance when analyzing polar molecules of biological origin. Moreover, CI in practice has offered greater sensitivity.

3. Field Desorption Mass Spectrometry: Concerns for Thermal Decomposition

Electron impact ionization (EI) has been successfully used in analyses of organic compounds for more than 20 years. A major limitation in this application has been that each compound must have sufficient vapor pressure when being introduced into the mass spectrometer to obtain a mass spectrum. Since most compounds have to be evaporated from the solid or liquid phase in order to obtain the necessary vapor pressure inside the ion source, thermal energy is transferred to the molecules. In the case of thermally stable compounds, it is possible to evaporate the molecules without decomposition, followed by electron impact ionization. The resulting mass spectra normally show molecular ions and fragment ions induced by electron impact. Often, the mass spectra are characteristic for the investigated compounds and can be used for identification and for structural analyses of unknown compounds. In most of these cases, the molecular ion has sufficient intensity to allow the determination of the elemental composition of the molecule by exact gas measurement using a high-resolution mass spectrometer.

Many organic molecules decompose, however, at least partially or even completely during electron impact ionization because of the energy transfer during the ionization process. Therefore, in the case of complete decomposition, the molecular ion cannot be detected in the electron impact mass spectrum. Frequently, the use of a suitable ionization process, whereby less energy is transferred to the molecule, allows the detection of the molecular ion. Field ionization has been successfully applied to such problems for about 10 years.[705] [706]

Recently, chemical ionization (CI) has been introduced as a further gentle ionization method.[707] The ionization is caused by ion molecule reactions inside the ion source and a relatively high pressure of about 1 torr. Using this ionization procedure, considerably stronger molecular ion groups of thermally unstable molecules are obtained compared to electron impact.

Both mentioned ionization procedures, field ionization and chemical ionization fail however, in those cases in which compound decomposition is caused by the energy necessary to convert the compound to the vapor phase. A significant breakthrough in this problem has been given by the introduction of a special field desorption technique four years ago.[708] Applying this method, a great number of polar compounds of low volatility were investigated and this was the first time that mass spectra were obtained without thermal decomposition.

Field ionization of a molecule occurs in the gas phase in a very strong electric field (about 10^7 volts/cm), a few angstroms distance from the so-called field anode or field ion emitter. The energy transferred to the molecule during this ionization process is negligible compared to 70-volt electron impact ionization. Prior to field ionization, only the thermal energy required for evaporation is transferred to the molecule.

Removal of an electron from a molecule by high electric field is based on the quantum mechanical tunnel effect. In the presence of a high electric field, the atom potential will be deformed in such a way an electron finds a potential wall of finite thickness. This wall can be under-tunneled with a certain probability. If the molecule is field ionized from the absorbed state and desorbs as an ion, one calls this phenomenon field desorption (FD). In practice the sample is deposited onto the field anode itself in contrast to the field ionization technique where the sample has to be evaporated, for instance, via a direct insertion probe. The field desorption takes place at considerably lower temperatures than are normally required for the evaporation of a molecule. Since little thermal energy is transferred to the molecule, and because of the low energy transfer and the ionization procedure itself, molecular ions of extraordinary intensity are formed. This is valid even in the case of very polar and highly unstable compounds.

Thus, it can be seen that biological materials which for physiological fluid transport are frequently polar can now be approached structurally in a very direct manner, making use of the extreme sensitivity of mass spectrometry.

4. Field Ionization (FI)

Field ionization, like field desorption mass spectrometry, relies on extremely high electric fields acting on atoms or molecules to produce positive ions based on the quantum-mechanical tunneling effect. This process, which is basically an evaporation of positive ions over a potential barrier on the surface due to the strong field, usually results in small pulses of ions. Continuous ion current results when field ionization occurs in the gas phase at or near the emitter. The ion formation results from a strong interaction between the field and the outer electron shell of the neutral molecules. Field ionization using a multipoint source has been developed at Stanford Research Institute[709,710] and M. Anbar has utilized this instrumentation and isotopic dilution analysis to measure low levels of nicotine in blood. This method was submitted to the National Cancer Institute and claims a sensitivity of 0.1 ng/sample with only 0.1 ml of blood and a precision of 1%. Briefly, 200 ng of d-3-nicotine (deuterium labeled) is added to the blood sample. The nicotine is separated by TLC and the spot identified on a fluorescent plate. The silica layer of the spot is scraped off and introduced into a heated inlet system of a non-fragmenting FI mass spectrometer.

The ratio of the ion currents from mass 162 (nicotine) and mass 165 (d-3-nicotine) indicates the concentration of nicotine in the blood sample relative to the known concentration of d-3-nicotine. Estimated cost per sample is $10 or less. This technique appears promising and warrants further study. The major drawback appears to be the time required for separating the nicotine via TLC.

5. Single- or Multiple-Ion Detection: Sensitivity Maximization

When using gas-liquid chromatography combined with mass spectrometry, identification can be made from retention times as well as from the mass spectrum of each peak obtained as it elutes from the column in a repetitively scanning mass spectrometer. In a repetitively scanning mass spectrometer, however, the basic sensitivity of the instrument is sacrificed in order to register meaningful signals over a wide range of masses in a very short time: i.e., production of a mass spectrum of a peak as it elutes from the column. Under these conditions the data produced by the mass spectrometer required a frequency response of 1-10 KHZ to transmit the signals to the amplifying system without skewing or clipping. Single-ion monitoring (for specific ions known to occur in biogenic amines and amino acids) has allowed sensitivities to 10^{-15} moles or the femtomole level.[711]

Multiple-ion monitoring provides a somewhat greater specificity with little loss in sensitivity and is achieved by alternating the accelerating voltage to focus only the ions of choice on the collector slits. Operation of a mass spectrometer in one of the above modes is clearly one of the major approaches to gaining high sensitivity.

C. GAS-LIQUID CHROMATOGRAPHIC DETECTORS

During the past decade a corpus of analytical methods has been developed, based upon gas phase analytical procedures. At present techniques involve principally gas-liquid chromatography for purposes of separation and estimation, mass spectrometry for identification and structural studies[7,12] and computer techniques for handling instrumental data. These methods use microgram and submicrogram samples and highly complex mixtures can often be analyzed directly. The utility of mass spectrometers as sensitivity detectors has been discussed in the previous section and the costs of this instrumentation can be considerable, depending on the degree of sophistication desired.

The development of liquid phases for chromatography of gaseous components has shown steady progress in terms of stability and selectivity; and newer materials are being developed for broader application. These trends will not be considered in this report.

Sample detection of gas chromatographic effluents has advanced considerably, however, and a number of detectors are now available which allow specificity and sensitivity for small increases in price. These adjuncts to the gas-liquid chromatographic technique are considered below.

Most commonly, quantitative identification of an eluted species is based on its characteristic retention volume as indicated by an essentially non-discriminating thermal conductivity or flame ionization detector. However, many samples, particularly those originating from biological investigations, contain so many constituent compounds that the resulting chromatogram is a complex maze of peaks. Often the analyst is interested only in a few of these peaks and is faced with the problem of determining which they are and how he can eliminate interferences from nearby overlapping, or even obscuring, peaks. Flame photometric detectors (FPD) are one of the most common selective detectors in use today. It is essentially a flame photometer and responds to the presence of a characteristic element or group present in the eluted species. The eluted species passes into a flame (usually H_2/O_2) which supplies sufficient energy first to produce atoms and simple molecular species and then to excite them to a higher energy state. The excited atoms and molecules subsequently return to their ground state with emission of characteristic atomic line spectra or molecular band spectra. By monitoring a selected emission wavelength, a phototube signal reproduces the chromatographic peak of interest.

Detection limits in the subnanogram range are often possible because all of the energy available in the flame can be utilized for the atomization and excitation processes since none is required for vaporization of the sample. Commercial flame photometric detectors rely on a narrow band pass filter to isolate the appropriate analytical wavelength range.[7,13]

The most highly developed flame photometric detectors are selective for phosphorus and for sulfur. These elements are detected by monitoring narrow band emissions from the simple molecular species HPO and S_2 at 526 and 394 nm, respectively. Detection limits are normally about an order of magnitude lower than can be achieved for these compounds with a flame ionization detector.

The major field of application of gas-liquid chromatography-flame photometric detectors has been in the determination of pesticides and pesticide residues containing sulfur and phosphorus. The flame photometric detector has found wide application in detecting gaseous sulfur compounds in air.[714-715]

The performance of the detector designed by Stevens et al.[714] has been reported to possess detection limits of 2 ppb for H_2S and 4 ppb for SO_2.

Quantitative determination of sulfur compounds in gas phase cigarette smoke using flame photometric detection of gas chromatographic effluents has recently been applied by Horton and Guerin.[716] These workers quantitated levels of COS, H_2S, CS_2 and SO_2 and brought out interesting problems of sulfur quantitation in the presence of metal surfaces and chromatographic packings.

Another interesting detecting device that deserves consideration here is the microwave plasma detector which is based on essentially the same spectroscopic principle as the flame photometric detector since both monitor a characteristic high-temperature line or band emission. However, the selective detection of metal chelates and organic derivatives of toxic metals such as organo-mercury compounds provides a capability beyond the flame photometric detector. In the microwave plasma detector, however, the relatively low energy flame of the flame photometric detector is replaced by much higher energy microwave plasma excitation. The plasma is established by irradiating an inert carrier gas, such as argon or helium, flowing through a tuned microwave cavity. Free electrons in this plasma acquire sufficient kinetic energy from the electromagnetic field in the cavity to cause fragmentation of molecular species eluted into the plasma and to excite these fragments. The observed spectra arise principally from either diatomic molecules or free atoms, although emission also results from some recombination reactions in the plasma. The microwave plasma detector offers a high degree of selectivity for a wide variety of elements including carbon, fluorine, chlorine, bromine, iodine, phosphorus, and sulfur with detection limits commonly in the nanogram or picogram ranges.

The main limitation of the microwave plasma detector is due to widely distributed spectral interferences which necessitates the use of a monochromator for wavelength selection in place of the simpler narrow bandpass fitters employed in the flame photometric detector.[717]

The majority of applications for selective detectors can currently be satisfied by the flame photometric detector and the higher cost, and greater complexity of construction and operation have minimized its use in analytical gas chromatography. The future success of this detector lies in the determination of metal chelates and organo-metalics as mentioned above.

A modification of the conventional flame ionization detector (sometimes called the thermionic detector or the alkali flame ionization detector) has allowed enhanced sensitivities about 1000 times greater for phosphorus compound with a modest increase in price. The sensitivity is achieved by placing with precise accuracy a pellet of alkali metal salt composed of KCl, RbCl, Rb_2SO_4, Ha_2SO_4, CsCl, or CsBr[718] at the flame tip. The mechanism by which this modification has increased sensitivity has been discussed in considerable detail by Brazhnikov et al.[719]

The literature survey (Section III) has shown many nitrogenous compounds as constituents in mainstream cigarette smoke and these compounds characteristically give a diminished flame ionization detector response. However, the Coulson conductivity detector provides the best sensitivity and selectivity for nitrogen compounds and its application for these constituents can be recommended. The detector operates by converting the analyte to an ionic species whose conductance is monitored in a dc conductivity cell from which the analyte ions are continuously removed so that the detector has a differential, rather than an integral response. Although this detector has found utility for nitrogen compounds, different modes of operation enable detection of compounds containing chlorine and sulfur also.[720]

The electron capture detector is probably the most successful element selective detector with excellent exclusion characteristics for saturated hydrocarbons. The detector consists of a radioactive source (^{63}N or ^{3}H) which ionizes the carrier gas to produce a standing current. When an electronegative species is introduced into the detector, it reduces the current and is thus detected. The extent of current depends both on the number of electron-capturing species present and on their electronegativity, so that the electron capture detector is strictly selective for highly electronegative species, e.g., halogens, oxygen, and unsaturated groupings.[721,722]

Neelakantan and Kostenbauder[723] have utilized the selectivity and sensitivity of the electron capture detector in detecting as little as 0.03 picomoles of nicotine. The procedure is based on the catalytic hydrogenation of nicotine which yields two secondary amines, (N-methyl-4-(3'-piperidyl)-n-butylamine), which can be pentafluoropropylated. The introduction of these ten fluorine atoms takes advantage of the halogen sensitivity and detectability obtained from the electron capture detector. The application of this methodology to the determination of nicotine in blood has not been established.

D. HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

High pressure liquid chromatography, a rapidly developing field in separation sciences, holds great promise for resolving complex biological mixtures. Sample detectability of column eluents offers serious limitation to separation monitoring, however, and this, combined with our goal to consider methods which provide a new handle or better wedge into the analytical problems of smoke intake quantitation, was the reason for not considering HPLC in this report.

E. RADIOIMMUNOASSAY

Pioneering work by Yalow and Berson,[724] who first announced a radioimmunoassay procedure for the detection of insulin, initiated the rise of a new era in endocrinology which has yet to reach its zenith. Applications of radioimmunoassay or related techniques, such as competition protein binding, radioenzymatic assay, radioreceptor assay, and immunoradiometric assay give some indication of the broad utility of this method. The materials being assayed can include protein and non-protein hormones, vitamins, nucleic acids, enzymes, drugs, metabolites, cancer antigens, viral antigens, antibodies, and structural proteins. Any substance which is of sufficient low concentration to make detection by biochemical, chemical, or instrumental means difficult, if not impossible, is a candidate for this type of assay.

Radioimmunoassay is based on the principle that a compound to be measured and the same compound labeled with a radioactive tracer compete for binding to, or reaction with, an antibody. This competitive principle is quantitated by determining the amount of radioactivity bound and free. Thus, the ratio of bound to free radioactivity decreases as the concentration of the measured non-radioactive compound increases. The antigen used for radioactive labeling must be pure for the assay to be specific, but for immunizations this is not an absolute requirement because the assay depends on competition between standard antigen and labeled antigen. Thus, antibody binding sites directed against other moieties do not enter into the reaction.

For the production of antisera rabbits have been used most frequently because they generally combine small animal advantages with that of providing ample quantities of serum. Radiolabeled antigen preparation sometimes introduces a difficult problem in synthesis and must be considered in the overall assessment of methodology. High specific radioactivities are important when preparing antigen to achieve maximum sensitivity. This is necessary because the concentration of labeled antigen must be kept below the minimum concentration of antigen to be measured.

The application of radioimmunoassay to smoke related problems has been introduced by Langone et al.[725] These workers developed radioimmunoassay procedures for nicotine and cotinine and have provided a

new approach to quantitating these smoke components in body fluids to assess smoke intake. Although the method is capable of estimating levels of nicotine and cotinine to *p*mole levels in tissues and physiological fluids, they have not been able to correlate levels of nicotine or cotinine with smoking history. Whether the lack of correlation is related to analytical methodology, pharmacokinetics of nicotine or an inability to control smoke intake is not known.

It is certain that radioimmunoassay, or related methods of competitive protein-binding assay will play a larger role in quantifying trace components from physiological fluids. The degree of success it offers for smoke intake problems may depend in large part upon recognition and control of various biochemical variables involved in dosimetry. This approach would offer direct measurement and would not require use of any additions to the tobacco; e.g., markers, isotopes, etc.

F. STABLE ISOTOPES

The major advantage of isotopes as tracers lies in the very close similarity of their chemical and physiochemical properties to those of the most abundant isotopes and the ability to discriminate between the two using currently available methods of extremely high sensitivity. Stable isotopes have the further advantage of being relatively safe to handle and of having infinite lifetime.

The determination of the relative abundances of stable isotopes usually is accomplished by mass spectrometry. A major requirement is that the isotopically enriched sample must be sufficiently volatile or must be capable of being converted to a volatile form in order to be analyzed by the mass spectrometer. Under the proper conditions, abundance measurements are reproducible to approximately 0.1%. Fortunately, most measurements involve relative isotope abundances between some standard and the sample, resulting in better accuracy. Modern isotope ratio mass spectrometers employing double collectors and a dual inlet system can achieve an accuracy of better than 0.005% through comparison with a standard.

Stable isotopes have been used in a wide variety of applications, most of which involve their use as tracers either to follow the fate of a particular atom in a reaction series or an entire molecule in a complex system. For example, tracing particular atoms of a molecule is vital to the elucidation of the origin of certain atoms of cellular metabolites in biosynthetic studies[726,727] or in the study of reaction mechanisms where the fate of one or more atoms is diagnostic of a particular mechanism.[728,729]

Specific isotopic labeling is also used when the fate of the entire molecule is of interest, such as in studies of the metabolism of drugs or other compounds in living cells[730] or in isotopic dilution experiments in which quantitative analyses are sought.

Direct analysis of stable isotopes involves measurement of ion intensities of the several isotopic species of a molecular or fragment ion formed in a mass spectrometer. In addition, if structural information is required, a study of fragmentation reactions through the use of normal and specifically labeled compounds may also be necessary. The alternative method for the mass spectrometric analysis of stable isotopes is indirect and involves the combustion of the sample to a gas, usually H_2 for deuterium analysis, N_2 for ^{15}N analysis, and CO_2 for ^{13}C and ^{18}O analysis, followed by measurement of the isotope ratios of these gases.[731]

Probably the most significant aspect of stable isotopes (other than the safety factor) for our interests is a technique called isotope dilution analysis which provides a means by which a compound can be quantitatively determined in a mixture of chemically similar compounds by the isolation of only a small sample of the substance to be determined; i.e., large losses in the purification process are allowed. The technique is applicable to any substance that can be labeled by an isotopic marker, stable or radioactive.

To use methods involving isotopic markers, specially treated cigarettes are needed. This requires uniformly adding the label to the samples of various brands of cigarettes. One or two packs would be given to the subject to establish the level of smoke actually retained as a function of the number of cigarettes consumed per day.

G. SPECTROSCOPY

1. Introduction

Much of our current understanding of the fine scale structure of matter and of how matter interacts with electromagnetic energy comes from spectroscopy, the study of the characteristic frequencies at which resonating atoms and molecules emit and absorb radiation. Recently, there has been a virtual renaissance in generating and measuring extremely narrow resonance lines at wavelengths longer and shorter than those of light. This revival of interest was instigated, in part, by the passing of the Clean Air Act of 1970 and the publishing by the Environmental Protection Agency of national ambient air quality standards.[732,733]

The miniaturization of electric components, e.g., solid-state amplifiers and detectors, has made possible the development of compact and inexpensive optical monitors. Moreover, the availability of tunable visible and infrared lasers has had great impact in areas of chemiluminescence detection, infrared absorption techniques, and the remote sensing of gaseous components over a long optical path.

The spectroscopic techniques considered in this report are those that provide an added dimension in sensitivity and/or specificity which should be considered in measuring smoke related materials. None of these newer techniques have been reported in the cigarette smoke related literature.

2. Chemiluminescence

Light accompanying a chemical reaction is known as chemiluminescence and the occurrence of such phenomena in nature has been called bioluminescence. The reaction usually occurs during an exothermic chemical reaction in which some of the excess energy causes the excited electronic and vibrational states of the reaction products to become populated. This excited state must be capable of emitting a photon itself or transferring its energy to another molecule that can emit.

The efficiency of chemiluminescence is proportional to excited state production [(number of molecules going to excited state/number of molecules reacting) times the efficiency of emission (number of photons emitted/number of molecules in the excited state)] and for bioluminescence this value approaches unity but rarely exceeds 0.01 for chemiluminescence.

The application of chemiluminescence techniques to analytical problems has only recently been used for gaseous components[734,735] and the overall number of methods is small because of a lack of available reactions. And, in fact, those reactions applicable to smoke intake measurement may be limited only to sulfur and phosphorus compounds and the oxides of nitrogen. However, those methods that have been developed are quite successful because they are low-cost, sensitive, and specific.

Gas-phase chemiluminescence of sulfur and phosphorus arises from the recombination of species generated in a flame when such compounds are burned in a hydrogen-rich flame. For sulfur:

$$S + S = S_2^*$$

$$S_2^* = S_2 + h\nu \ (350\text{-}450 \text{ nm})$$

the chemiluminescence consists of a series of evenly spaced bands[736].

Phosphorus emission comes from POH, presumably due to a reaction between the hydrogen atoms and PO, to give the peak emission bands at 526 nm. The sulfur and phosphorus bands can be resolved by use of interference filters and detected simultaneously with two detection channels. Instrumentation and application of specific detectors for sulfur and phosphorus compounds, as covered previously under Section C, as well as gas-liquid chromatography -- flame chemiluminescence technique.

Gas-phase chemiluminescence techniques for the detection of O_3 and oxides of nitrogen have recently undergone extensive development.[3] The reaction of NO and O_3 produces near-infrared chemiluminescence.

$$NO + O_3 = NO_2^* + O_2$$

$$NO_2^* = NO_2 + h\nu \ (0.6 - 3.0 \ \mu m)$$

Construction of a detector to capitalize on this reaction was demonstrated by Fontijn et al.[737] and has been used to measure total oxides of nitrogen by means of prior conversion of NO_2 to NO.[738]

With cutoff filters required to eliminate interfering emissions, reduced pressures, and properly cooled photomultiplier tube, this method can detect 0.001 PPM NO along with a linear dynamic range of 10^7 up to 10,000 PPM. Nitrous,[739] nitric,[740-746] and nitrogen dioxide[740,744,746] have been observed in cigarette smoke, as well as numerous sulfur components,[743,747-758] the majority of these analyses being qualitative in nature.

3. Infrared Absorption

Infrared vibrational-rotational spectrum includes one or more distinctive absorptive bands for every gaseous component observed in cigarette smoke[759] and recent improvements in instrumentation have made possible a great increase in sensitivity of this type of infrared analysis. Fourier transform analysis has been most universally applied and recent improvements have been discussed by Hanst et al.[760]

Although infrared absorption can be utilized for characterization of numerous cigarette components, non-invasive breath monitoring appears most promising using the non-dispersive infrared analyzer.[761,762]

Several new types of non-dispersive analyzers have been developed and the instrumental design varies with changes in infrared source or type of detector used. Three of these variations will be discussed here for they are representative of the advances made which have markedly enhanced one or more of the following parameters: sensitivity, selectivity, and/or stability, and may be useful to estimate smoke intake using certain gaseous compounds.

a. Infrared Dual-Isotope Fluorescence Analyzer

The carbon monoxide analyzer constructed by Andros Inc.[763] utilizes energizing radiation consisting of two very narrow selective infrared spectral lines, one exactly matching the vibrational-rotational absorption lines of CO for sensing CO concentration, the other generated from CO^{18} which serves as a reference.

By use of a rotating filter, alternate pulses of these emitted spectral lines are passed through a single sample cell. Since naturally occurring CO contains 99.8% of the CO^{16} isotope, the CO^{16} lines will be absorbed by the CO of the sample cell and the CO^{18} lines provide a base line reference signal. This internal standard overrides transmission and drift problems. The performance characteristics include a noise level of 0.05 ppm, sensitivity below 1 ppm and accuracy better than 0.1 ppm.

b. Laser Spectroscopy

In conventional infrared spectroscopy, blackbody sources and grading or prison monochromators have been used to provide beams of narrow spectral width. These sources cannot provide sufficient power over a narrow enough spectral range to give adequate sensitivity and specificity.

Molecular gas lasers are not continuously tunable but can be made to emit in a number of discrete wavelengths corresponding to transitions between various inverted energy levels in the laser gas. An emitted wavelength is selected by rotating a diffraction grating, which forms one end of the optical resonator, and thus lasers can serve as a light source in a non-dispersive analyzer if the laser emission line falls on one of the absorption lines of a component to be measured.

More importantly, by using a pulsed laser or by chopping a continuous laser beam at audio frequency, a sound wave is produced which can be detected by a pressure transducer or microphone. This effect, called the "optoacoustic effect," was discovered by Bell,[764] Röntgen[765] and Tyndall[766] and is discussed in a later section.

Tunable lasers are not often found in analytical laboratories and many of the anticipated applications have yet to be realized. This can be attributed to many causes, including the rather high cost associated with the present commercial systems, inconvenience in using many lasers over even a modest spectral range, the difficulties in setting up an automated programmable wavelength sequencing or scanning system, and the normal delays in developing and accepting new methods. However, two tunable laser systems are finding a rather broad acceptance in spectroscopy and these are the dye laser and the diode laser. By a combination of techniques these lasers are continuously tunable in the infrared and visible region covering a major portion of the spectrum of interest for analytical chemists. These will be discussed individually below.

(1) Tunable Dye Laser Spectroscopy

The tunable dye laser produces very high intensity, coherent, collimated, spectrally pure light pulses of nanoseconds duration[767,768]. The dye laser is based upon the principle that lasing action can be obtained from an organic dye solution by irradiating the dye medium with an extremely intense optical source of short duration. This irradiation produces a population inversion for the molecular energy levels of the dye, so that the dye solution is then capable of exhibiting optical gain as dye molecules undergo stimulated emission. By placing the dye within a dispersive optical cavity, a tunable coherent output of narrow bandwidth is obtained. The dye cell is placed between a reflecting grating and a partially transparent output mirror to form the optical cavity. Selection of a precise single frequency out of the wide range available from a dye is done by a series of prisms and coupled short cavities. The selected single frequency can be smoothly tuned along the dye laser's range using an automatic feedback loop. Laser Energy, Inc. (Rochester, New York, 14625), and GCA/McPherson (Acton, Massachusetts) have constructed commercially available dye lasers which are tunable from approximately 360-640 nm by a combination of various dyes. This range is dependent upon several factors, such as the dye and wavelength region selected. The UV dyes are less efficient than the visible dyes, and as a result lower peak powers are observed. The output power will drop off as the fringes of the dye's tuning range are approached. However, since the dyes exhibit overlapping, this falloff can be minimized. The peak power of a sealed dye solution will vary with the pulse repetition rate.

Resolution studies have shown that bandwidths of about 1 nm are obtained from this monochromator cavity arrangement. Since the nitrogen laser's pulse duration is only about 10 nsec,[769,770] lasing action of the dye within the dye cavity will be limited to very few passes between the output mirror and the grating. As a result, a bandwidth of about 1 nm is expected.[771,772] Both companies have chosen the pulsed molecular nitrogen laser as the pumping source which produces an intense pulse of UV radiation at 337.1 nm. This radiation is produced as a result of a fast transverse discharge, causing direct electron impact excitation of the nitrogen.

(2) Semiconductor Lasers and Emitters for the Infrared

Introduction

Injection lasers and light emitting diodes have been fabricated from a wide variety of semiconductors and are now available and cover a large portion of the spectral region from about 0.6 μm to beyond 30 μm. Of interest is a particular class of semiconductor emitters, the Pb-salt tunable diode lasers, which span the range beyond about 3.5 μm. These lasers cover a spectral region which is highly significant from the viewpoint of species identification by infrared signature and possess other properties such as tunability, high-brightness, ease of modulation and rapid turn-on and turn-off times which, altogether, give them a wide range of application. Many of these applications impact very directly on segments of the infrared industry.

Material Considerations

Figure 2 shows the dependence of laser emission frequency on composition for several important Pb-salt compounds. As seen in this figure, any desired emission wavelength from less than 3.5 μm to beyond 30 μm may be selected by controlling crystal composition. Pb-salt single crystals may be grown from the melt by the Bridgman and Czolchralski techniques and from the vapor phase by various methods. The source is a polycrystalline ingot of the desired composition sealed in an evacuated ampoule. The sodium heat pipe furnace liner insures that growth occurs under essentially isothermal conditions. This method of growth provides excellent control of crystal composition, hence of laser emission frequency, and yields well-faceted crystals especially suited to laser fabrication.

A distinctive feature of the Pb-salt semiconductors is that deviations from stoichiometry have a strong effect on the type and concentration of charge carriers in the crystals. An excess of the metal or non-metal constituent effectively dopes the crystals n-type or p-type, respectively. For most compositions of interest it is possible to establish desired carrier concentration levels and form pn junctions by adjusting deviations from stoichiometry, without adding foreign impurities.

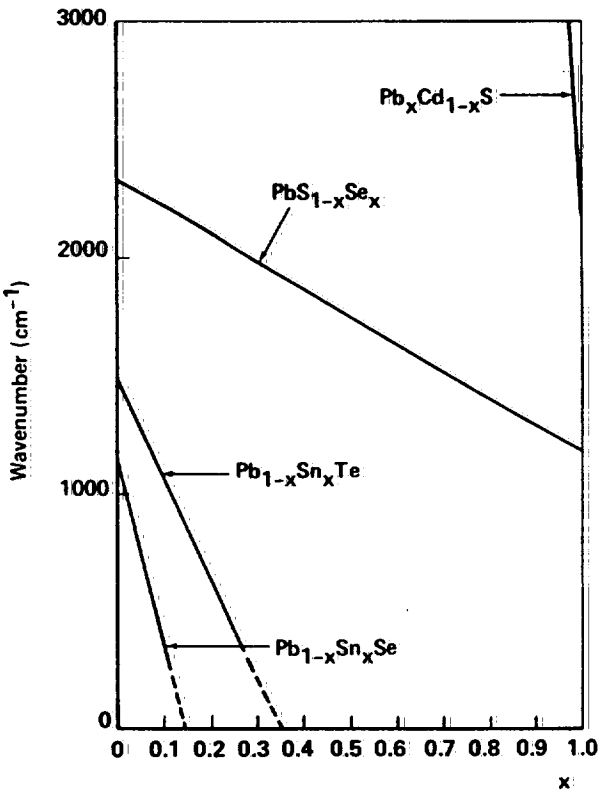


Fig. 2: Composition dependence of emission frequency for Pb-salt diode lasers. The solid lines indicate regions where laser action has been experimentally verified.

Lasers are shaped by cleaving and polishing operations and mounted on Cu heat sinks as shown schematically in Figure 3. Gold and In layers provide ohmic contact to the p- and n-surfaces, respectively, and In also serves as the bonding material. A buffer metal such as Pt between the Au and In layers eliminates effects associated with a low temperature Au-In eutectic. The pn junction depth is carefully controlled and is an important parameter in determining the bias current tuning rate. Typical dimensions are 500 μm between laser end faces, 250 μm width and 200 μm between the metallized surfaces.

Operating Characteristics

Laser emission occurs when forward bias current through a diode laser exceeds its threshold current. Threshold currents are strongly dependent on temperature, as illustrated in Figure 4, for a $PbS_{1-x}Se_x$ laser fabricated in our laboratory. CW operation for this laser was possible for temperatures up to 65 K.

Power output from a $PbS_{1-x}Se_x$ laser manufactured in our laboratory is shown in Figure 5. These data were measured with pulse bias to eliminate heating at the higher current levels. CW power outputs up to approximately 2 milliwatts could be achieved with this laser. The linear behavior of the power versus current curve is an indication of gain saturation and consequently of high internal quantum efficiency. Recently, a group at MIT Lincoln Laboratory has developed new fabrication techniques for PbS lasers, and has achieved CW output powers for this material as high as 360 milliwatts (4.3 μm emission wavelength)[773].

The emission spectra of Pb-salt diode lasers typically exhibit one or more modes with mode spacing in the order of 0.5 cm^{-1}. The width of a single mode is generally 3×10^{-5} cm^{-1} or less. Figure 6 shows, as an example, a PbSe diode laser spectrum measured with a grating monochromator; the indicated linewidths are determined by the monochromator resolution. The laser of Figure 6 was operated in a magnetically tuned system.

The emission frequencies of Pb-salt diode lasers can be tuned by a variety of methods. The simplest method in practice is current tuning, in which variation of the bias current changes the diode temperature by a small amount. Single mode tuning ranges are typically 1 - 5 cm^{-1} and overall tuning ranges 10 - 40 cm^{-1} for current tuning. With the addition of a magnetic field of up to 50 kOe the tuning range can generally be extended to at least 50 cm^{-1}, and gaps between modes effectively closed.

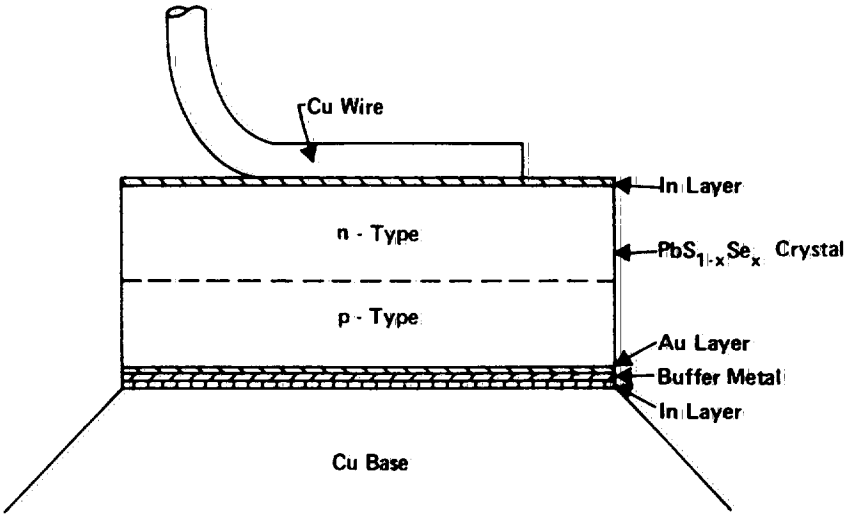


Fig. 3: Conceptual diagram of a tunable diode laser. The left- and right-hand edges of the crystal define the Fabry-Perot cavity.

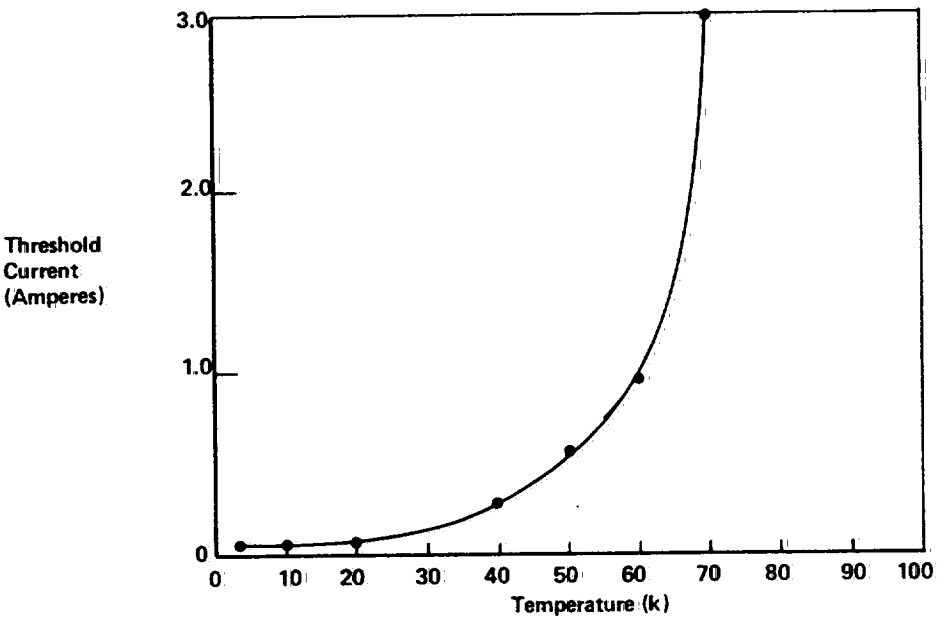


Fig. 4: Temperature dependence of threshold for a Pb-salt diode laser. The data were measured in the pulse mode with a frequency of 1 kHz and a pulse width of 20 microseconds.

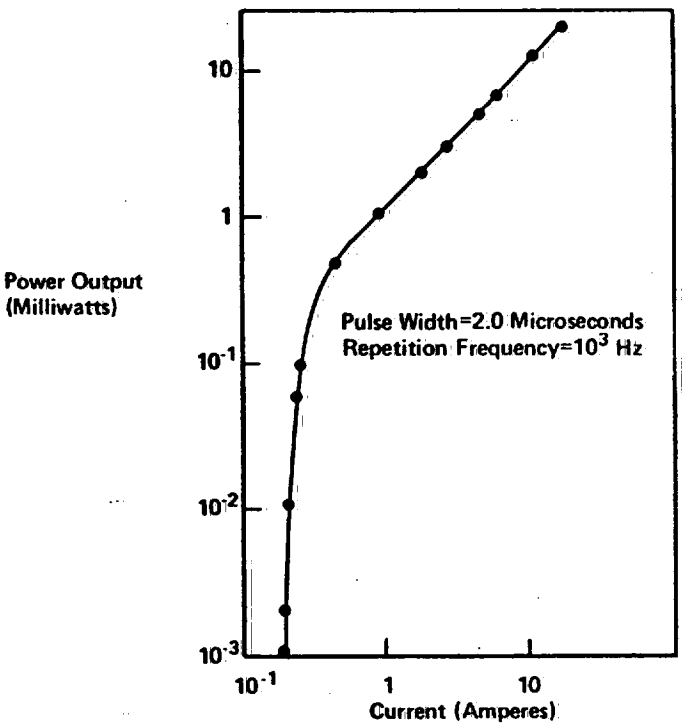


Fig. 5: Current dependence of output power for a Pb-salt diode laser. Note the linear dependence above about 0.5 amperes.

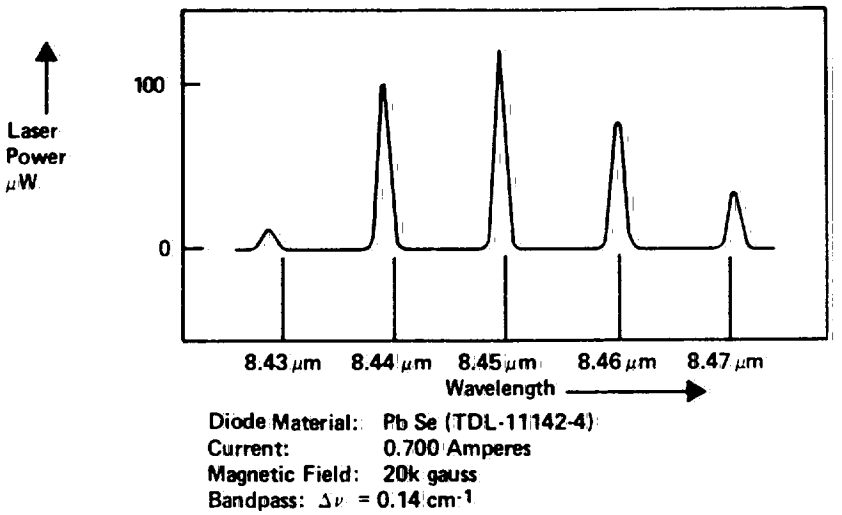


Fig. 6: Emission spectrum of a PbSe laser. The laser was installed in a magnetic field tuning instrument.

Tuning with hydrostatic pressure has been accomplished using a PbSe laser operating in the pulse mode at 77 K, and an overall tuning range of 915 cm^{-1} (7 - 22 μm) was achieved.[774] Temperature variation can generally be utilized to tune Pb-salt diode lasers over a range of 200 - 300 cm^{-1}. However, technological complexities have so far prevented pressure and temperature tuning from being viable methods for application.

Application of Tunable Diode Lasers

This section considers the main application areas which appear to be emerging for tunable diode lasers and discusses specific tunable diode laser instruments in use or in the planning stage. The applications can generally be classified into five areas: ultra-high resolution spectral measurements, sensing and monitoring instruments based on infrared absorption, heterodyne detection instruments, frequency response characterization of infrared systems and components, and isotope separation by selective optical excitation. The remainder of this section will consider these application areas in more detail.

Ultra-High Resolution Spectral Measurements

In this application the tunable diode laser beam is passed through a sample under investigation and the attenuation of the beam by the sample is monitored as the laser frequency is varied. The operation of tunable diode laser spectroscopic instruments is similar in concept to that of many conventional instruments in that they generate infrared probe radiation whose wavelength is scanned across a spectral region of interest. However, the resolution attainable with the laser source is determined by the laser linewidth which is 3×10^{-5} cm^{-1} or less; this represents an improvement in resolution over that of the best grating spectrometer or interferometer of two to three orders of magnitude, and opens entirely new areas of spectroscopic research. The high brightness of the laser source provides excellent signal-to-noise performance for such measurements and permits rapid spectral scanning. Tunable diode laser instruments are expected to have a revolutionary impact in many areas where spectroscopic measurements are of importance.

The feasibility of tunable diode laser spectroscopic instruments has been amply demonstrated by work at MIT Lincoln Laboratory during the past few years.[775] The Lincoln Laboratory group has studied spectroscopic features in a variety of substances that are completely inaccessible to conventional measurement techniques. Tunable diode laser spectroscopy has now been performed at other laboratories; workers at NASA Langley Research Center, for example, have recently reported on an extensive study of SO_2 with PbSe diode lasers.[776] Using a combination of magnetic and current tuning, this group obtained essentially continuous spectral coverage from 7.9 μm to 8.5 μm. Figure 7 shows an example of the results obtained by the NASA group.

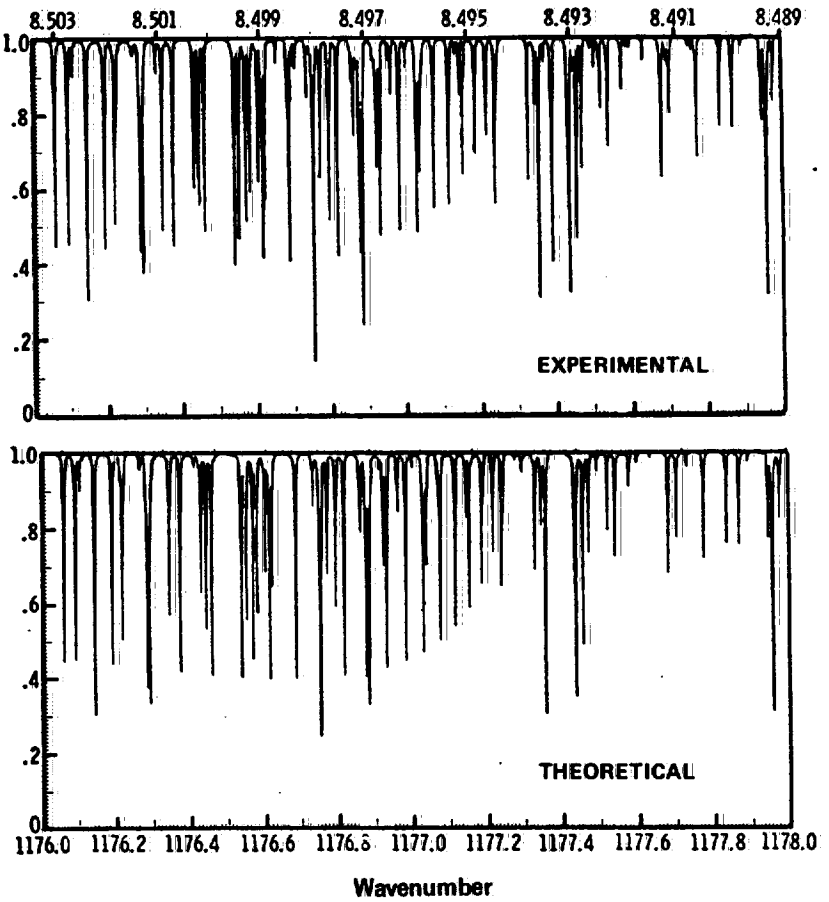


FIGURE 7: Absorption spectrum of SO_2 at a pressure of 1 torr. The upper curve was obtained from a PbSe diode laser scan, while the lower curve was theoretically generated from molecular parameters.

The experimental results are, in general, in excellent agreement with the theoretically generated spectrum. "Extra" lines observed experimentally can be ascribed to impurities in the sample cell (such as H_2O, which may account for the pronounced extra line near 1177.4 cm^{-1}) to hot bands.

Most gases possess identifying spectral absorption features accessible to tunable diode lasers. One can detect or monitor the concentration of such gases in spatial regions of interest by measuring the degree to which diode laser radiation is attenuated at identifying wavelengths; gases with similar absorption features can be separately identified by careful choice of absorption lines. This principle forms the basis for a variety of diversified sensing and monitoring instruments.

Many such applications are based on spectral measurements in the atmosphere under standard conditions of temperature and pressure. The linewidths of atmospheric broadened lines are in the order of 0.1 cm^{-1} which is actually within the resolving capability of high quality conventional spectrometers using thermal sources. However, the power available within this spectral band is, typically, orders of magnitude higher for TDL instruments than for conventional spectrometers.

In general, sensing and monitoring instruments should provide reliable unattended operation with a minimum of downtime and maintenance. As a consequence, cryogenic cooling required for the diode lasers and detectors in such instruments must be obtained from mechanical, closed cycle refrigerators such as those manufactured by Cryogenic Technology, Inc. Recent experiments have demonstrated the feasibility of this approach.

Because of its spectral purity, laser radiation may be used to selectively excite one substance in a mixture of two substances whose absorption spectra differ by a very small frequency increment. An example of such a mixture is that of two molecular gases differing only in the isotopic mass of one of the elements making up the molecule.

Selective optical excitation has potential application in separating isotopes. Figure 8 illustrates conceptually one possible separation method based on this mechanism. This figure shows for simplicity an idealized system consisting of a ground state E_0, an excited state E_1 (E_1') and an ionization continuum beginning at E_2. In part (a), the molecule is excited into level E_1 by radiation of frequency ν_{10} and then ionized by radiation of frequency ν_{21}. The ionized molecules may then be swept out by an electric field. In part (b), E_1' is slightly higher than E_1 because of an isotopic mass difference, hence the first excitation cannot occur, and ν_{21} is then not high enough to ionize the molecule. Thus, in principle, a separation of substances differing by isotopic mass may be affected by optical means.

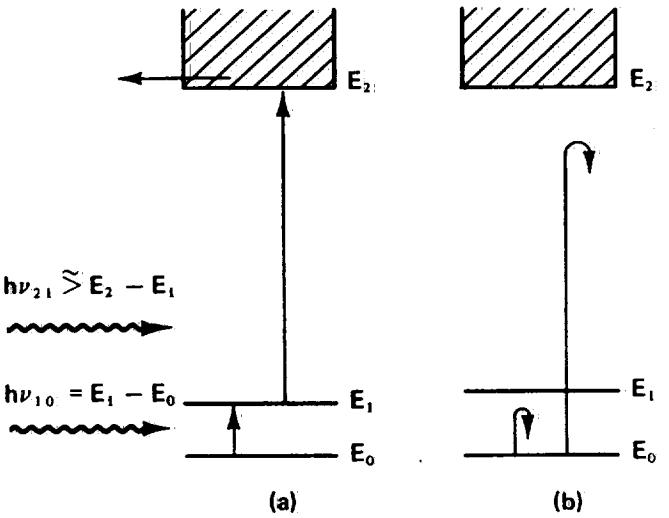


FIGURE 8: Conceptual diagram illustrating isotope separation by selective optical excitation. The molecule of (a) is ionized by the incoming radiation, whereas that of (b) is not.

Tunable diode lasers will find application in acquiring spectral information concerning isotopically differing substances, in process control or monitoring of isotope separation processes, or in supplying infrared power for an isotope separation process.

(c) Acoustic Amplifier

The optoacoustic effect has proved an effective method in detecting weak infrared absorptions[777,780] while avoiding excessively long path lengths.

A properly constructed sample chamber with microphone and pre-amplifier combination will detect gas concentrations as small as 1 ppb.[781]

Investigations of gaseous samples using the laser optoacoustic spectrometer have demonstrated a background signal arising from spurious scattering and absorption by the optical windows and surfaces of the cell.[782] In order to achieve the noise-limited sensitivities reported in this paper, the authors found it necessary to subtract this background signal from the measured signals. Since the background was reported to be 125 times the noise level, measurements had an accuracy of little better than one part in a hundred.

A modification of this sytem to offset the above limitation has been introduced by Dewey et al.[783,784] in two fundamental ways. First, a sample cell has been designed as an acoustic resonance chamber so that pressure fluctuations produced by spatially and temporally non-uniform excitation contribute to standing acoustic waves within the chamber and secondly, the excitation beam was so modulated that its frequency was coincident with one of the natural resonant acoustic frequencies of the chamber. These two developments have added significantly to the analytical capability of trace gas detection by laser-optoacoustic spectroscopy.

VI. SMOKE INTAKE QUANTITATION: MOST PROMISING METHODS

VI. SMOKE INTAKE QUANTITATION: MOST PROMISING METHODS

A. INTRODUCTION

The goal of this study was to find a component of mainstream smoke which would show a quantitative relationship with smoke intake when extracted from body fluids, or alternatively, induced some physiological or biochemical manifestation. The conclusions reached from the study are based on our understanding of the capabilities and limitations of current analytical methodology (Section V), considerations influencing smoke intake measurement (Section IV), and a consideration of the potential usefulness of each of the mainstream smoke components, as compiled by the literature search (Section III).

Although many smoke components were found in biological fluids and biological effects were shown to occur with smoking, this deductive approach was encumbered by a preponderant void in quantitative data, especially in relation to total smoke intake or cigarettes consumed. On the other hand, recent developments in spectroscopy (Section V,G), mass spectrometry (Section V), and the utility, as well as availability, of stable isotopes (Section V,F), have provided an additional dimension to non-evasive, highly specific, sensitive analysis and the opportunity to establish baseline values for quantitation of smoke intake.

To this approach we have selected two compounds to serve as indicators of smoking. The compounds are stable isotopes of carbon monoxide (representative of gas phase compounds) and water (representative of compounds found in both the gaseous and particulate phase), respectively of mainstream smoke. We expect transfer of these materials from smoke to body fluids to be most direct and offer the least complications from their metabolic fate. Having satisfied these prime considerations, analytical methodology, and/or instrumentation capable of providing the sensitivity and specificity demanded was investigated. It is our goal in this section to define in some detail factors and concerns which we have considered in developing this methodology. These approaches, in our judgment, appear most promising for providing quantitation to the difficult problem of smoke intake.

B. CARBON MONOXIDE AS A MARKER FOR SMOKE INTAKE

1. Exogenous Carbon Monoxide

Addition of exogenous CO to the human body and removal of CO from the body takes place almost exclusively in the lungs with very little transport of CO through the skin.[785] Since the inspired CO passes through the lungs and circulates in the blood as COHb, the measurement of COHb provides an accurate method for assessing individual CO exposure occurring in a previous 15-hour interval.[786-788] Measurements of COHb in different occupational groups have shown significant differences;[789] e.g., vehicle-related occupations -- metal, chemical, stone and glass processing, printing, welding, electrical assembly and repair -- all indicated higher CO exposure, while students, housewives, and groups associated with mental health, library science, religion, art, and entertainment had lower concentrations of COHb. Non-smoking New York cab drivers showed a mean COHb saturation of 2.5 percent with a range from 1.3 to 5.8 percent while off-duty drivers ranged from 1.0 to 1.5 percent, with a mean of 1.2 percent. The percent COHb saturation for smoking cab drivers in the same city had a mean of 6.9 with a range from 3.0 to 13.0 percent.[790]

Thus, different occupational groups, as well as urban or suburban living introduce considerable variability in body CO as determined by COHb measurement. Carbon monoxide in expired air has also served as a means of assessing body exposure to the gas and a number of workers have demonstrated a linear correlation between COHb and CO in expired air.[791-794]

2. Mainstream Cigarette Carbon Monoxide

Carbon monoxide (CO) is one of the most abundant constituents of tobacco smoke (single puffs of 35.0 ml contain approximately 1.6 mg[795] and of all the cigarette-related literature, except for nicotine, no single cigarette constituent has received greater study (Section III). Much of this interest is rather recent due to an awareness that chronic adverse effects can occur with CO at levels less than those needed to give rise to toxicity. These toxicities are well recognized and manifest themselves in several ways: first, CO and O_2 react in a competitive manner for the same ligand binding sites on the hemoglobin molecule (hemoglobin's affinity for CO is 218 greater than for O_2,)[811] and second, COHb interferes with the release of the oxygen carried by the hemoglobin molecule.[812-815] Secondly, several reports have considered carbon monoxide in relationship to pathogenesis of coronary heart disease,[796,797] myocardial infarction,[798] and selected dysfunctions of the nervous system.[799-801] Thus, carbon monixide is increasingly being considered, along with "tar" and nicotine as the compounds in cigarette smoke which most likely give rise to the health hazard of smoking.

Inhalation of mainstream cigarette smoke has been reported to result in retention of smoke particulates in the lung of about 70% while retention of the vapor phase varies with the chemical and physical properties of individual gases. Approximately 55% of the carbon monoxide is retained but 99% of the nitrogen dioxide is retained even with slight inhalation.[802-803]

3. Endogenous Carbon Monoxide

The earliest indication of mammalian production of CO was that of Roughton and Root[804] who found small but measurable amounts in normal human blood. Confirmation of endogenous production of the gas was reported by Sjöstrand[805] when he observed higher concentrations of CO in expired air than in inspired air. He estimated the body production of CO in these non-smokers to be approximately 0.5 to 1.0 milliliter/hour. Sjöstrand further demonstrated CO production was greater in patients with hemolytic anemia, extensive trauma after certain surgical procedures and blood transfusions.[805-806] Later, it was suggested that in vivo production of CO originated from the one-carbon fragment at the alpha-methene position of bilirubin. This was confirmed and amplified by Ludwig, Blakemore and Drabkin in 1956, using labeled heme.[807]

The measurement of endogenous production of CO is now recognized to be clinically useful in the diagnosis of hemolytic states. However, studies by Coburn et al. have shown the rate of CO production in normal man to be 20 to 30 percent greater than the rate calculated from circulating red-blood-cell hemoglobin.[808-812] This was consistent with earlier studies[813,814] which had shown sources of bile pigment distinct from the breakdown of heme derived from circulating red-blood-cell hemoglobin. This was again amplified by a number of workers[815-819] who have demonstrated that the non-hemoglobin hemes are important sources of bile pigment which is estimated to represent about 40 percent of the total endogenously produced CO.[820]

A summation of carbon monoxide metabolism, distribution, and excretion (modified slightly from that of Coburn[808] is presented below in Figure 9. These, along with variations in exogenous sources, offer considerable analytical concern for meaningful measurements of expired carbon monoxide, especially at low levels. For these reasons, the stable isotopes are recommended, thus obviating the problem of endogenous and exogenous carbon monoxide. The following pages contain prices and companies which sell the stable isotopes that could be used in these experiments.

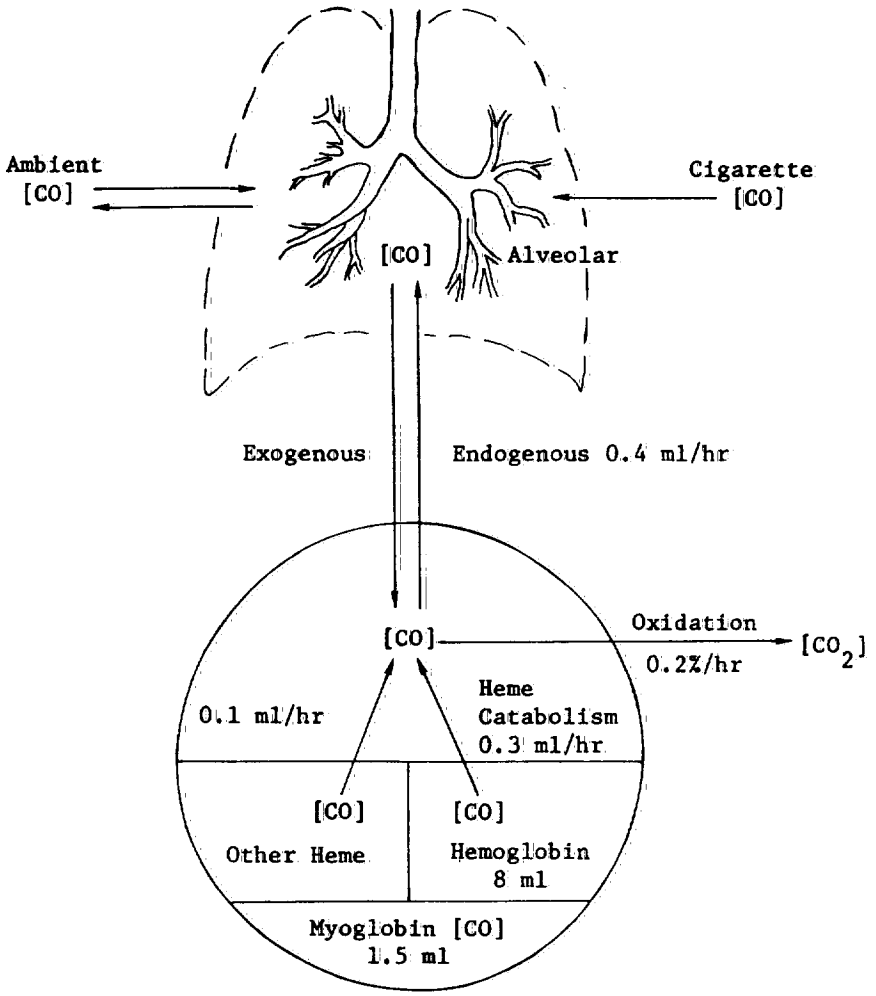


Fig. 9: Sources of Carbon Monoxide and Distribution in the Body

TABLE 5

STABLE ISOTOPES OF CARBON MONOXIDE

ISOTOPE	VOLUME	PRICES 90 atom %	
^{13}CO	100 ml	$ 40*	37**
	250 ml	90*	76**
	1000 ml	245*	215**
$C^{18}O$	100 ml	290*	185**
	250 ml	500*	370**
	950 ml	950*	665**
$C^{17}O$	only in 10 atom %		
$^{13}C^{18}O$, $^{13}C^{17}O$	Not commercially available but may be obtained by custom synthesis		

* Prices from company No. 6 (see below)

** Prices from company No. 1 (see below)

Companies Offering Stable Isotopes

1. Miles Laboratories, Inc.
   Kankakee, Illinois 60901

2. Schwartz Bioresearch, Inc.
   Orangeburg, New York 10962

3. Mound Laboratory
   Miamisburg, Ohio 45342

4. Oak Ridge National Laboratory
   Isotope Sales Dept., Oak Ridge, Tenn. 37830

5. Merck, Sharp and Dohme, Inc.
   Teterboro, N. J. 07608

6. Bio-Rad Laboratories
   Richmond, Calif. 94804

7. International Chemical and Nuclear Corp.
   Chemistry and Radioisotope Division
   Irvine, Calif. 92664

8. Prochem Limited
   Lincoln Park, N. J. 07035

9. Koch Isotopes
   Cambridge, Mass. 02139

C. WATER AS A MARKER FOR SMOKE INTAKE (ALTERNATIVE METHOD)

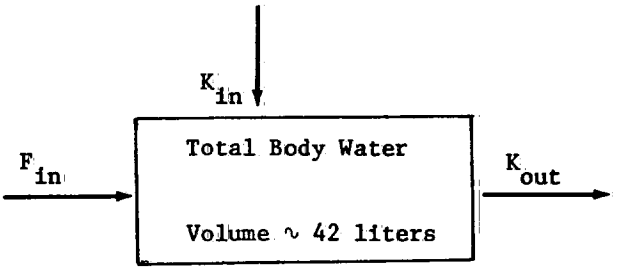
Using criteria set forth in Section IV, a review of the list of chemical agents reported in smoke revealed water under certain conditions as a possible alternative marker to the more obvious carbon monoxide. In part this selection is based on knowledge that little of the water load by whatever route of administration is metabolized and that the major portion of the water administered distributes uniformly in total body water, although a small fraction of the water from smoke will be incorporated into cells and tissues and fixed. Water present in smoke should completely equilibrate with water in plasma and bone within four hours. This rapid equilibration should permit reliable estimates of the added water from smoke from samples of virtually any body fluid.

In view of the relatively large amount of endogenous and exogenous water available from sources other than from cigarette smoke, it is obvious that ordinary water cannot be used as a marker and that some means of quantitatively differentiating water from cigarette smoke from water from all other sources is needed. The use of water labeled with the stable isotope, deuterium (D), was therefore considered.

The natural abundance of deuterium is 0.0156% although this hydrogen to deuterium ratio (H/D) can vary somewhat depending upon the source of the water. The adult male has a total body water ranging between 50% and 73% of body weight, while in the female the total body water ranges between 44% and 65%. For the male, total body water is 40 to 45 liters. The amount of body fat greatly influences the proportion of total body water.

Although the use of D_2O to measure total body water is well known, it has not been used in measuring smoke intake. The feasibility of using D_2O for smoke intake requires knowledge of the amount of D_2O that can be incorporated into cigarette smoke, the increase in D_2O levels in body water above background resulting from smoking, the sensitivity of the instrumentation needed for analysis, and knowledge of the pharmacokinetics of water. Where uncertainties exist in the amount of D_2O which can be incorporated into the smoke and the relationship to daily cigarette consumption, a matrix of pertinent variables was made and the level of D_2O in body fluid calculated for each different set of conditions.

The following model is used to describe the transfer of D_2O from smoke into total body water. With increasing dose (or smoking) the level of deuterated H_2O rises exponentially to the point where the eliminated deuterated H_2O equals its input.



where x = amount of deuterium as D_2O and DHO (in mg) in body water,
x_o = background deuterium,
V = volume of total body water,
F = input of deuterium as D_2O and DHO,
K_{out} = elimination of water as a percentage of body water,
$\frac{x}{V}$ = concentration of D in body water,
K_{in} = consumption of water as a percentage of total body water.

$$\frac{d\frac{x}{V}}{dt} = \frac{F}{V} - K\frac{x}{V}$$ Concentration of D for elimination (1)

where $\frac{F}{V}$ = input concentration/day

(2)

$\frac{Kx}{V}$ = elimination concentration/day

The amount of D_2 in body water compartment after start of inputting F is

$$X = x_o + \frac{F}{K}(1 - e^{-kt}) \quad (3)$$

The concentration in the elimination is

$$\frac{x}{V} = \frac{x_o}{V} + \frac{F}{VK}(1 - e^{-kt}) \quad (4)$$

Thus, the following relationship:

(1) Increasing the daily dose of D_2O increases the concentration of deuterium proportionally (Figures 10, 11).

(2) Increasing the elimination rate K shortens the time to reach the plateau and lowers the level.

(3) The level will reach 98.1% of the plateau at a time

$$t = \frac{4}{K} \text{ and } 95\% \text{ at } t = \frac{3}{K}$$

(4) The decay rate for flushing out the deuterated water is 5% per day of the level above background; i.e., 63% flushed out in 20 days and 98% after 80 days.

Assumptions used to evaluate D_2O and body fluid as a means of estimating smoke intake:

(1) Sixty kg subject with a total body water volume of 42 liters.

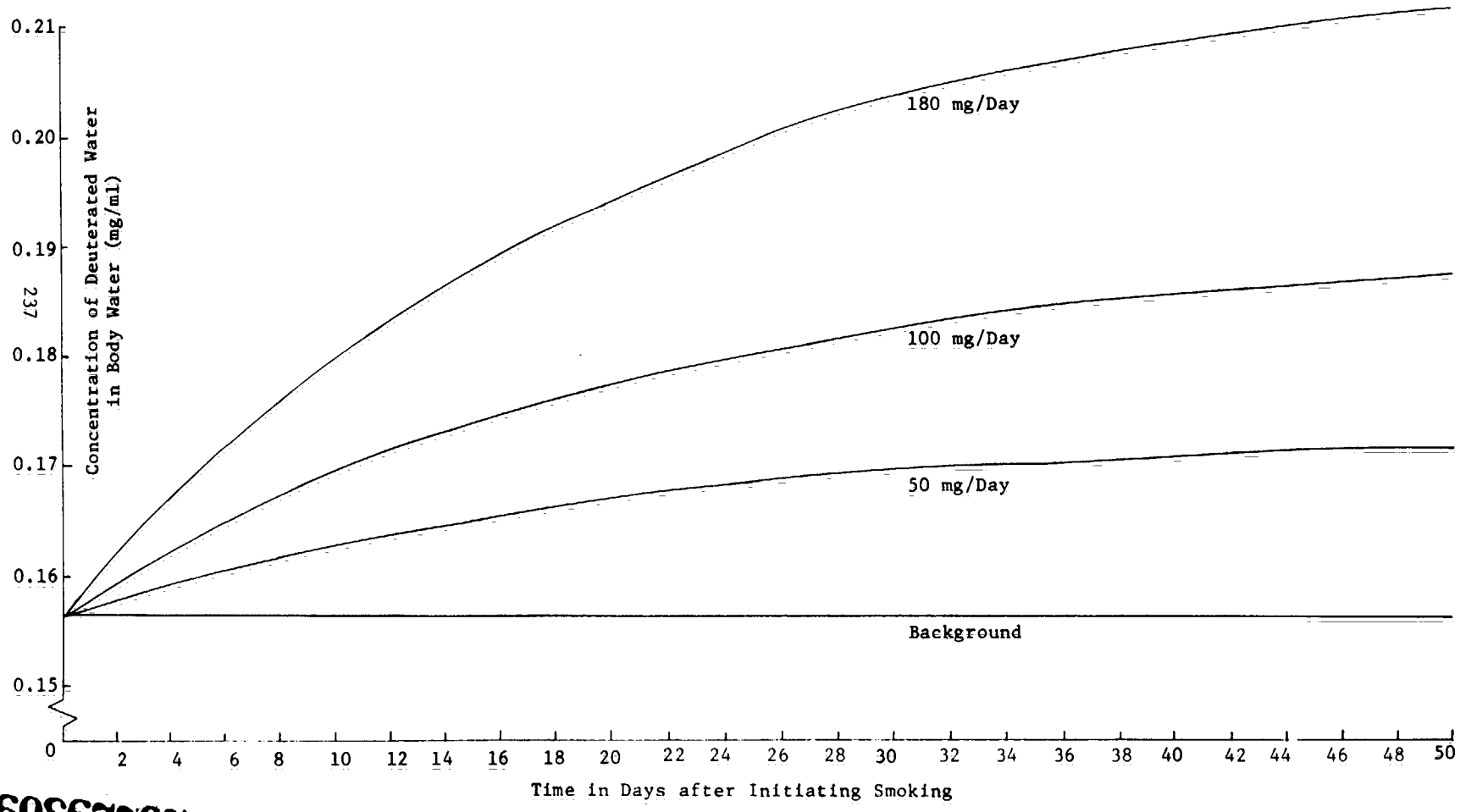
(2) The normal background ratio of H to D in water is 0.015%. Thus, there is a total of 6.3 g of D in the total volume of water or 0.15 mg D/ml.

(3) Seventy percent of deuterated water from cigarettes enters and equilibrates with body water.

(4) Water clearance rate via urine, sweat, expired air, etc., is estimated at 5% per day (K = 0.05).

The tables and charts contain data relating the total amount of deuterium in body water as functions of the smoking habit: i.e., depth of inhalation of the smoke and number of cigarettes per day. For example, in order to reach a concentration of deuterated water of 0.1896 mg/ml at equilibrium, an individual would have to receive an average of about 100 mg of deuterated water per day. One can receive 100 mg of D_2O from several different smoking modes: e.g., deeply inhaling the smoke from five cigarettes, moderately inhaling the smoke from ten cigarettes, or occasionally inhaling the smoke from twenty ciagarettes. Thus, knowing the concentration of deuterated water at equilibrium and the total number of cigarettes smoked, one can estimate the smoking habits of any individual. Further if a sufficient number of individuals are studied, one might demonstrate that people who fall into the high medical risk group exceed a certain concentration of deuterated water, whereas others in the low risk group fall below this same concentration. This information may be useful in better characterizing the hazards of smoking.

Fig. 10: CONCENTRATION-TIME CURVES FOR VARIOUS INPUTS OF SMOKE CONTAINING DEUTERATED WATER



Arthur D Little Inc

Fig. 11: CONCENTRATION-TIME CURVES FOR VARIOUS INPUTS OF SMOKE CONTAINING DEUTERATED WATER

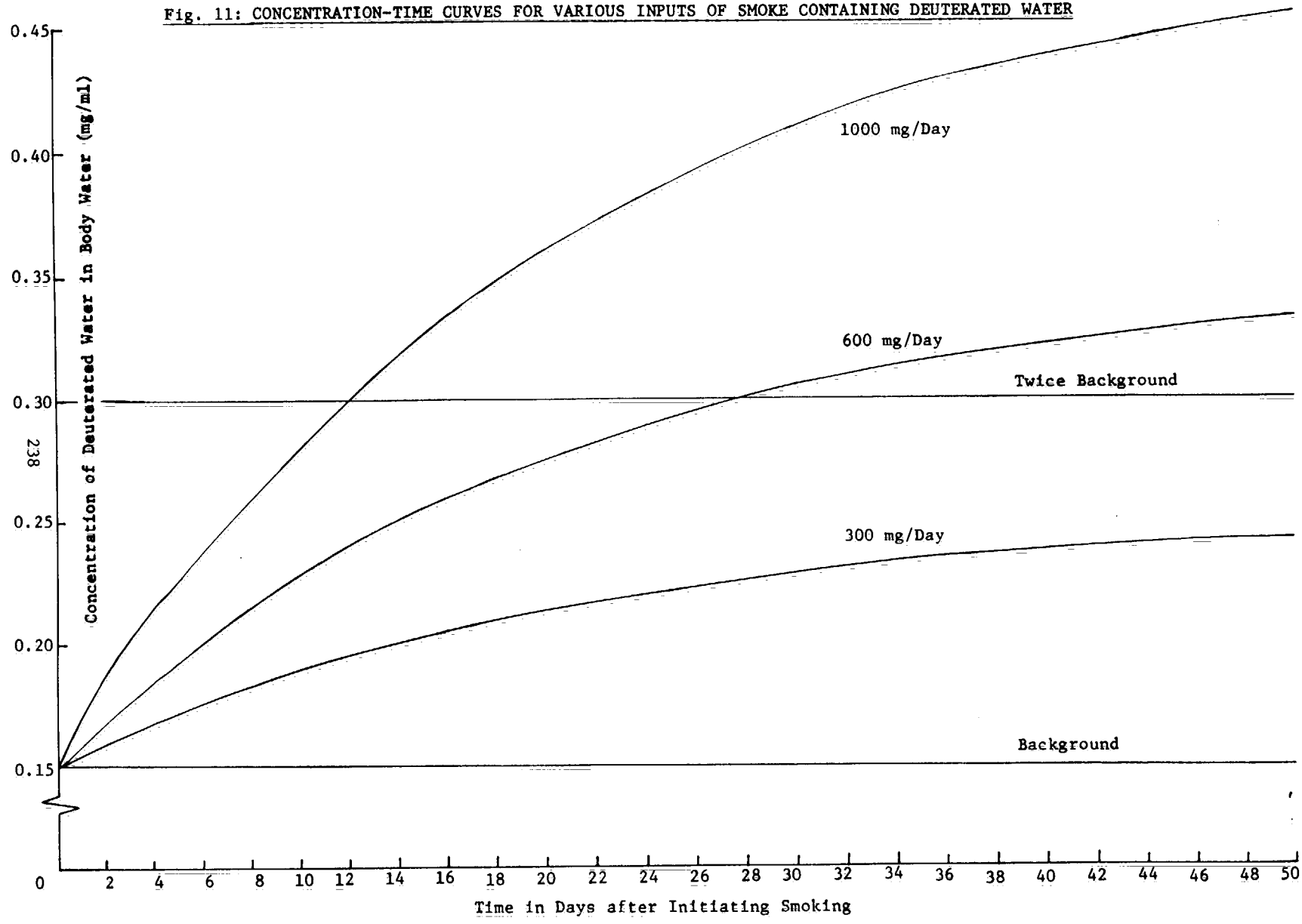


TABLE 6

THE INTAKE OF DEUTERATED WATER AS A FUNCTION OF SMOKING HABITS

Deuterated Water Inhaled/Cigarette (mg)	Total Daily Intake of Deuterated Water from Smoke (mg/day)			
Cig/day	5	10	20	40
25	125	250	500	1000
20	100	200	400	800
15	75	150	300	600
10	50	100	200	400
5	25	50	100	200
1	5	10	20	40

TABLE 7

CONCENTRATION OF DEUTERIUM IN BODY WATER WHEN EQUILIBRIUM IS ESTABLISHED AS A FUNCTION OF SMOKING HABITS

Deuterated Water Inhaled/Cigarette (mg)	Deuterium Concentration in Body Water - mg/ml			
Cig/day	5	10	20	40
25	0.1980	0.2396	0.3230	0.4896
20	0.1896	0.2230	0.2896	0.4230
15	0.1813	0.2063	0.2563	0.3563
10	0.1730	0.1896	0.2230	0.2896
5	0.1646	0.1730	0.1896	0.2230
1	0.1580	0.1596	0.1630	0.1696
0	0.1563	0.1563	0.1563	0.1563

D. STANDARDIZATION OF SMOKING AND SMOKE INTAKE - PILOT STUDY

In view of the large number of variables inherent in the smoking of a cigarette, it is essential that every effort be made to accurately control the amount of smoke inhaled. With active human or animal puffing this is not possible. For this reason the problem of accurate smoke generation can best be handled by a mechanical device or smoking machine. In contrast to virtually all previous attempts at quantitating smoke intake where smoking was not controlled, use of a smoking machine permits accurately measured amounts of smoke of more uniform and known composition for selected compounds, e.g., nicotine, carbon monoxide, acetaldehyde, etc.

The next major obstacle to reliable and accurate estimates of smoke intake is a measure of the actual retention. The error attributable to uncertainties in the retention of smoke can be minimized by having the subject inhale on "cue" all of the smoke delivered from the smoking machine, pause in inhalation, then breathe several times into a suitable bag. The mouth is then rinsed several times with distilled water and the volume measured and recorded to determine retention in the mouth (important for water soluble gases and particulate materials). The difference between the amount of smoke inhaled as measured by the marker compound and the amount of marker recovered from the exhaled breath and mouth represents a reasonable approximation of the amount retained in the respiratory tract. For best results these experiments would undoubtedly have to be carried out with experienced smokers, and possibly with animals, e.g., tracheotomized dogs, prior to use of human subjects.

The labeling of all commercial cigarettes sold to the public with an appropriate isotope marker, i.e., carbon monoxide $^{13}C^{16}O$, and/or deuterated water D_2O, is virtually an impossible task. A reasonable alternative is to make available a sufficient number of cigarettes for each of the major brands of cigarettes with the appropriate markers or a marker releasing material to characterize a small but representative sample of the smoking population.

Initially, smoke intake measurements will be made with cigarette labeling carried out in the laboratory in order to assess the merits of the proposed approaches under controlled and idealized conditions. Factors as, degree of isotope incorporation into and release from tobacco, acceptability, taste, burning characteristics, hazards, in addition to evaluating the overall usefulness of the marker to measure smoke intake will all be examined.

Analysis of body fluid for the marker will be carried out simultaneously on samples of breath and blood to provide data on the quantitative relationship between concentration of the marker in blood and breath for carbon monoxide, or blood, breath and urine for deuterated water.

Subjects will be instructed to inhale puffs of smoke from cigarettes containing the isotope(s) generated by the smoking machine and to recover in a suitable vessel all smoke exhaled as well as rinsing of the mouth to recover materials retained in the mouth. After the completion of the smoking, the cigarette butt (3 cm) will be analyzed for TPM, nicotine, water, etc., to establish amount and composition of the condensate collected in the butt. The increase in amount of condensate, nicotine and other compounds will be related to the degree of puffing. Since these higher boiling point compounds will collect in appreciable amounts in the butt of puffed, but not in smoldering cigarettes, analysis of some of these compounds can provide a gross estimate of the degree of puffing vs. non-puffing of cigarettes. Use of filter cigarettes is recommended since the analysis of the filter is more reliable than the butt of the non-filter variety, and also because filter cigarettes now make up approximately 85% of the cigarettes sold.

A second measurement will consist of measuring directly from the smoking machine the total amount of CO or D_2O isotope in smoke from each cigarette. These values serve as a basis for calculating total input dose, i.e., the total amount of marker delivered from a single cigarette. This value is needed for calculating the proportion or fraction of cigarette smoke inhaled from analyses of body fluids as well as from calculation based on mass balance.

To sample the breath, after each puff and especially during the period after smoking, subjects should be instructed to breathe deeply several times into an empty inflatable bag. This procedure will insure more representative sampling of the marker concentration in the alveolar air.

Total urine samples will also be collected throughout the first week for D_2O analysis, then periodically until the level is reduced to those of the control period. Samples of body fluids will be analyzed for ^{13}CO and/or D_2O by appropriate instrumentation described in Section VI.

Based on published data on the size of the various body fluids the concentration in body fluid actually measured will be used to calculate the total body load. This will be compared with the amount retained using a mass balance. A second objective will consist of analysis of aliquots of body fluid collected after smoking in an attempt to characterize the rate of elimination and also to obtain a correlation between total amount of marker inhaled and the total amount excreted for various numbers of cigarettes smoked per day as well as for different numbers of days of smoking.

Successful demonstration of either ^{13}CO or D_2O as suitable markers of smoke intake will also provide a means of critically evaluating the effectiveness of other previously employed markers of smoke intake, e.g., CO, nicotine and thiocyanate, as well as others listed in the previous sections of this report. This evaluation would result from a comparison between levels in body fluid found with isotope markers and levels with normal smoke constituents. A final comparison would correlate for individual smokers the amount and concentration of various smoke condensates in the butt with the daily dose of smoke inhaled. If a significant correlation is found, an approximation of the smoke intake can be obtained during periods when body fluid samples are not available from analysis of components in the butt. Butt analysis would only be useful for smokers in whom the level of retention has been previously established and found to be relatively consistent.

E. STUDY OF INTAKE OF THE CHRONIC SMOKER

Successful demonstration in the pilot study of an appropriate reliable marker of smoke intake would justify enlarging the size of the smoking population. The nature of body fluid sample would depend on which marker was selected as well as upon whether an isotope marker was required or not. Should a normal smoke constituent be a suitable marker, the task is obviously considerably simpler than if a mass isotope must be used. The latter would require production of separate lots of cigarettes in sufficient quantity to satisfy the needs of NCI. This would best be handled by the cigarette manufacturers by virtue of their extensive experience in blending tobacco and tobacco additives.

Three or four packs of isotope containing cigarettes would be given to the subject after an appropriate number of body fluid samples are taken to establish baseline values. The subject would be instructed to smoke the usual number of special cigarettes in the normal manner. At daily intervals (during the early phases more frequent sampling might be required) the subject would either collect his own body fluid, e.g., urine, breath, or in the case of blood, go to a designated institution qualified to take blood.

From levels in body fluid samples during the early part of smoking, an estimate of smoke intake can be made from the rate of increase of the marker concentration in the sample as well as the level of the marker in body fluid once a plateau is established. Each of the special (label) cigarette butts would be saved by the subject and submitted for analysis to ensure that the cigarette was in fact smoked and also if possible to determine the degree of active puffing.

Prior to initiating these studies the effects of storage and packaging would have to be studied to ensure that the level of isotope in the cigarette is not altered during storage or during the time the package is opened for smoking.

F. EXPERIMENTAL DESIGN

1. Carbon Monoxide

a. Cigarette Labeling and Testing

Several compounds have been considered for cigarette labeling. The criteria of selection have been based primarily on the ability to give rise to a measurable and reproducible amount of CO on decomposition at a level that does not represent an additional hazard.

The choice of the CO liberating compound will be determined. Iron carbonyl is an example of such a material. Iron pentacarbonyl is an industrial product which is prepared by classical carbonyl synthesis from CO and finely divided iron according to the following equation:

$$Fe + 5CO \rightarrow Fe(CO)_5$$

The compound is a colorless liquid and decomposes at 130°C with a ΔH° of decomposition of 138.3 kCal for the reaction:

$$Fe(CO)_{5(g)} \rightarrow Fe_{(g)} + 5CO_{(g)}$$

If absorbed intact, iron pentacarbonyl has been shown to be toxic. Another possiblity is the iron tetracarbonyl, a stable compound under ordinary conditions having dark green prismatic crystals. The tetracarbonyl dissociates to CO and Fe at 140° to 150°C. Still another compound that shows greater sensitivity to heat, is the diiron nonacarbonyl ($Fe_2(CO)_9$), which is stable at room temperature in dry air and is soluble in ether, petroleum ether and benzene. On heating to 100-120°C the compound decomposes to the iron pentacarbonyl and CO as follows:

$$2Fe_2(CO)_9 \rightarrow 2Fe(CO)_5 + 2Fe + 8CO$$

The appropriate CO liberating compounds would require a synthesis involving the stable isotope of carbon monoxide (^{13}CO). The tobacco of such a cigarette would require uniform distribution of the compound containing the isotope and a constant rate of carbon monoxide release as the cigarette is smoked. The total yield of the labeled carbon monoxide should be preferably quantitative, reproducible and show insignificant changes with changes in combustion (Section IV).

b. Sampling and Method of Analysis

Expired air should be collected via rebreathing techniques in a gas sampling bag (Z), preceded by a dry ice trap to remove water vapor and higher molecular weight gases. Immediately after collection, the sampled volume is spiked with the stable ^{18}O-isotope of carbon monoxide. With this addition of the $^{12}C^{18}O$ stable isotope, further sample manipulation can be carried out without concern for quantitation.

(1) Laser Spectroscopy for Analysis of Carbon Monoxide (^{13}CO)

We have covered in considerable detail spectroscopic approaches in Section V, and more specifically the diode laser as a means of optimizing sensitivity and specificity. Our best evidence indicates that this type of instrumentation will be directly applicable to the analysis of ^{13}C-carbon monoxide in the expired air from smoking subjects. In this section we describe the instrumentation in detail and its sensitivity.

Lead salt tunable diode lasers offer 50-100 kHz resolution and are at least four orders-of-magnitude better than the conventional absorption spectrometer. The diode laser has the capability to adequately resolve Doppler broadened gas absorption lines which are typically 10-100 MHz wide. Various methods are used for tuning, including magnetic field, hydrostatic pressure variation, temperature and current tuning; some of these are used in conjunction.

CO has been investigated in detail with a $PbSe_{1-x}Se_x$ laser, using magnetic field tuning in conjunction with current tuning,[821] adjusted to obtain near-coincidence between the diode laser output and the absorption line. Fine tuning through the line was then accomplished by varying the current.

The model TDLS-II tunable diode laser spectrometer is an integrated system for ultra-high resolution.[822] The laser source achieves a spectral resolution of 3×10^{-5} cm^{-1} or better and represents an improvement in resolution over the best grating spectrometer or interferometer of two to three orders of magnitude. The high brightness laser source provides excellent signals-to-noise performance and permits rapid spectral scanning. Easily interchangeable laser sources permit tuning ranges greater than 50 cm^{-1} to be achieved throughout the infrared spectrum between approximately 3.5 μm and 30 μm.

(Z) Alltec Associates, 202 Campus Drive, Arlington Heights, Ill. 60004

A block diagram of the overall system is presented and the modules may be purchased as an integrated, pretested system, or the individual modules may be purchased separately.

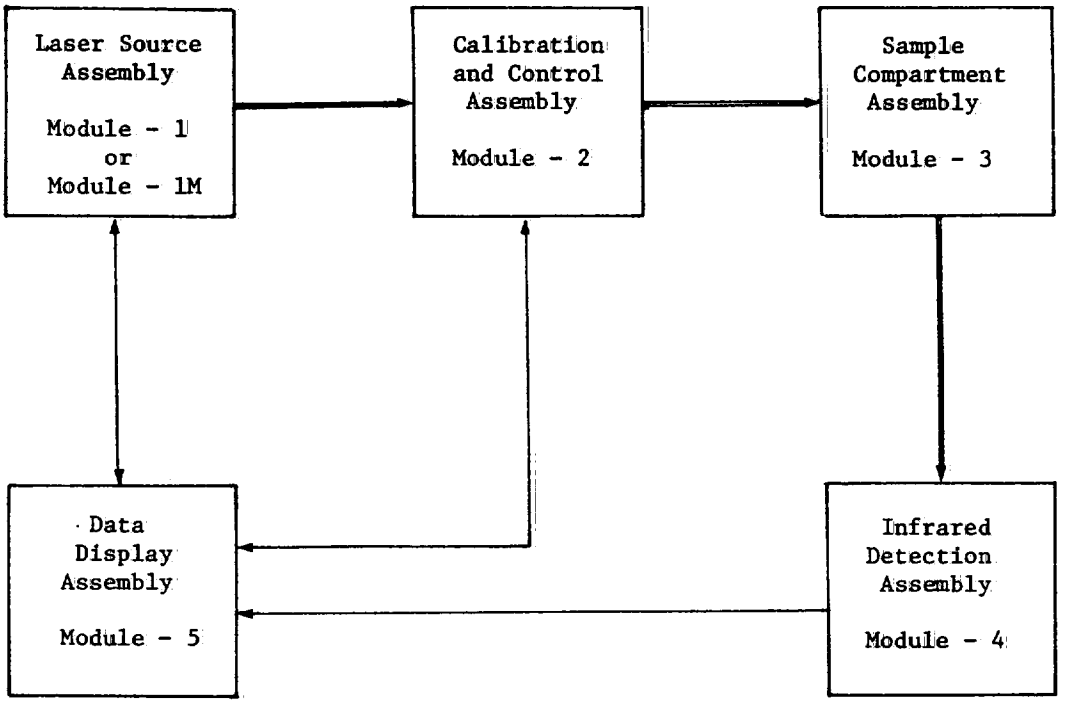


Figure 12: Model TDLS-II Modular Block Diagram

Laser Source Assembly

Module - 1:

Interchangeable tunable diode laser mounted in dewar
Liquid He dewar with liquid level indicator (4-hour hold time)
High stability laser power supply with automatic sweep and digital readout

Module - 1M:

Interchangeable tunable diode laser mounted in dewar
Liquid He dewar with liquid level indicator (8-hour hold time)
High stability laser power supply with automatic sweep and digital readout
50 kilogauss superconducting magnet mounted in dewar
High stability magnet power supply

Calibration and Control Assembly, Module - 2:

1/2-meter Ebert grating monochromator
Air-cooled blackbody source
Tuning calibration etalon
Collection and collimating optics

Sample Compartment Assembly, Module - 3:

Adjustable length working enclosure
Mounts for standard gas sampling accessories
Custom optics available

Infrared Detection Assembly, Module - 4:

Solid-state infrared detector mounted in dewar
Liquid N_2 or liquid He dewar
Matched solid-state preamplifier
Collection optics

Data Display Assembly, Module - 5:

Synchronous detection amplifier
XY recorder (11-in. x 17-in. display)
Rapid-scan oscilloscope monitor

The simplest configuration consists of a diode laser source assembly, mode selector, gas absorption cell, infrared detector and electronics. The lowest detectable concentration of CO is given by:

$$C_{min} \sim \frac{0.2}{L} \text{ parts per million}$$

where L is the length of the absorption path in meters. Using a commercially available 40-meter White cell would thus allow measurements down to about 5 parts per billion. Added sophistication could probably decrease this by another factor of 1,000.

The absorption lines of interest (^{13}CO) will occur around 4.9 μm, which is easily accessibly to diode lasers. This is also a region that is relatively free from interferences from other species.

(2) Mass Spectrometry; Isotope Dilution Analysis and Multiple Ion Detection (Alternative Method for CO)

As an alternative method to laser spectroscopy multiple ion detection and isotope dilution techniques provide an accurate and a most general analytical approach. Application of the isotope dilution methodology has been suggested by the Stanford Research Institute to Dr. Gori of the National Cancer Institute. In this work, Anbar et al.[6] have detected low levels of NO and nicotine, using a multipoint field ionization source.

For this study, we believe a similar approach should be considered with high-resolution mass spectrometry. A quantitative determination of the stable isotope $^{13}C^{16}O$ (28.9983 amu) in expired air can be achieved by the immediate addition of $^{12}C^{18}O$ (29.9992 amu) to the sample volume. Determination of these ion ratios with an alternating voltage accelerator would provide a precise determination of the carbon monoxide in the sample with a maximum of sensitivity.

The instrumentation involved in the type of analysis is commercially available from a number of firms and has been documented in the literature.

Both procedures are limited in their detection limits by the natural abundance of the isotopes in the sample. Thus, consideration should be kept in mind for using these isotopes which have the lowest natural abundance.

2. Mass Spectrometry; Hydrogen Isotope Ratio Determination for Deuterated Water Analysis

Deuterated water readily undergoes exchange with normal water present in the mass spectrometer inlet system and the isotope ratio will be affected by that of the previous sample analyzed. Experience has shown that molecular hydrogen (H_2) is the most suitable terminal intermediate in the analysis of deuterium and many methods have been developed for the combustion of an organic sample to water and the subsequent reduction of water to hydrogen gas. Prepurified water samples are passed over uranium foil in a quartz micro-converter tube kept at 600°C.[823] The hydrogen gas produced is collected in an automatic Toepler pump. The HD produced in the conversion is in equilibrium with hydrogen and deuterium according to the reaction:[824]

$$H_2 + D_2 \rightarrow 2HD \qquad K = 3.8$$

Mass spectral analysis presents an unusual difficulty in that four peaks are obtained due to the following ions:

Atomic Mass Unit	Ion Composition
$M/_e$ 1	H^+
$M/_e$ 2	H_2^+, D^+
$M/_e$ 3	HD^+, H_3^+
$M/_e$ 4	D_2^+

Ion-molecule reactions account for the H_3^+ ion and its abundance varies as the square of the pressure and hence, will be small at low pressures. Extrapolation to zero pressure gives the actual ratio HD/H_2. For most accurate results in the study of natural abundance, standards of precisely known ratios, approximating those of the sample, should be used.

VII. OTHER CONCEIVABLE METHODS

VII. OTHER CONCEIVABLE METHODS

Included in this section are methods, some of which have been used on laboratory animals or man and others that are at the conceptual stage. Each one cited has some merit in that it may be non-invasive, ultra sensitive, or merely because it represents a new and novel approach to smoke intake.

(1) "Use of Bromine-82 as a Measure of Cigarette Smoke Intake in the Dog" (Wehner[553]).

This personal communication includes results of experiments in which deposition of the neutron exposed cigarette smoke in the dogs was measured by whole-body counting and analysis of blood and excreta. Bromine-82 was the best suited tracer. It could be detected by whole-body counting for up to 9 days as compared to 3 and 4 days for ^{42}K and ^{24}Na, respectively. Blood levels of ^{82}Br could be monitored for 4 days. Excreta also showed detectable levels of ^{82}Br for 4 days. Between 30 and 70% of the ^{82}Br body burden occurred in the blood where it was retained for an appreciable period of time. The ^{82}Br blood level was a linear function of the number of cigarettes smoked whereas whole-body counts showed a decrease in Br, K and Na deposited per cigarette as more cigarettes were smoked.

The maximum total body radiation dose received by a dog smoking one neutron activated cigarette was less than $5 \cdot 10^{-4}$ rads which may make this technique applicable to studies with human subjects. Effective retention half-time largest for ^{82}Br was 30 hours which is about the same as the radioactive half-life, indicating minimal excretion. Use of radioactive cigarettes even at levels low enough to present little hazard would not be readily acceptable to use with the general public.

(2) "In Vivo Spectroscopy" (J. Young, Oak Ridge National Laboratory, Personal Communication).

Three variations of an in vivo determination of COHb (and possibly other compounds) are proposed.

a) Blood is drawn into a capillary tube in which COHb is immediately determined by spectrophotometric procedures.

b) A tunable laser impinges on a pyroelectric detector after passage through a proper path length of blood. The design could be incorporated into a 16- or 18-gauge needle which would only have to be inserted into a vessel to determine COHb.

c) A design is considered which could be incorporated into a hypodermic needle as in (b). It would use a tunable laser in the UV region of the spectrum and a fluorescence detector. The probe is cylindrical with a hemispherical end and a cylindrical hole at the end along the axis of the probe. Laser light directed down the probe axis would cause excitation and emission of light in the fluid within the hole. The light would be directed back due to the shape of the probe end to a detector at the base of the probe.

These approaches, especially variation (a), are reasonable approaches but need further study. The systems described in variations (b) and (c) require implantation of a needle or catheter into a blood vessel.

(3) "Ferromagnetic Contamination of the Lung" (D. Cohen[105])

Ferromagnetic particles have been found in the human body (e.g., asbestos in the lungs of asbestos workers, Fe_2O_3 particles in the lungs of arc welders, particles from food cans in the stomach). The presence of these particles was detected with a flux-gate magnetometer. The possible use of this device to study the distribution of inhaled air labeled with Fe_2O_3 (harmless) into the lung is suggested. It may also be possible to apply this technique to estimate the dose of smoke if smoke is found to have ferromagnetic properties or if individual smokers are given Fe or Fe_2O_3 containing compounds in labeled cigarettes. This approach is still at the laboratory stage of development.

(4) "Use of Fluorescent Histiocytes in Sputum" (Vassar et al.[536]).

This paper reports experiments in which fluorescent histiocytes were demonstrable in every sputum sample examined from people who smoked. A direct positive proportional relationship between number of cigarettes smoked per week and both the intensity of fluorescence in the cytoplasm and the number of fluorescent macrophages present in an average field of sputum smear was found. Some evidence suggests that dissolved polycyclic hydrocarbon components are the fluorescent material; polycyclic hydrocarbons such as 3-4-benzpyrene have a marked affinity for lipids such as are found in high concentration in the histiocyte cytoplasm.

(5) Ear or Surface Oximetry

Measurements of in situ oxyhemoglobin saturation have been made using commercially available electro-optical instrumentation. Because of the number of variables, measurement with whole blood, as with ear oximetry, is considerably more complicated and requires extensive extraction of desired signals from undesired signals to obtain desired accuracy. Problems involved in the transfer of light through the ear have been reviewed by Sutterer.[590] Based on conversations and written communication with Mr. Sutterer, the development of an electro-optical carboxyhemoglobin measuring instrument appears to be well within present technology. The principal problem is related to the non-specific absorption and diffusion of light due to presence of blood cells. The shape, size, concentration, flow rate, as well as pigmentation and thickness of the pinna of the ear are sources of variability. Many of the equations for the density of blood have been formulated or may be applicable for developing an on-line instrument to measure carboxyhemoglobin in real time.

From inquiries to establish other centers of expertise we learned that Hewlett-Packard Company was currently in the process of developing an instrument capable of accurately measuring oxygen saturation of blood by ear oximetry. A meeting was held at Hewlett-Packard to confirm the accuracy of information and also to inquire about possible modification of the instrument to measure carboxyhemoglobin saturation. It was learned that the instrument, an oximeter, is now in the final phases of development and plans call for marketing the instrument in the latter part of 1974.

For oxygen saturation measurements an earpiece is placed over the pinna and light is optically coupled to the main section of the instrument. Light is transmitted through the ear using eight different wavelengths to deal with problems of differences in skin pigmentation, thickness, light scatter, etc. A highly accurate 24 bit digital processor makes the necessary computation. In the opinion of the developers, with proper modification, it should be possible to program the instrument to measure carboxyhemoglobin saturation. The anticipated selling price for the oximeter is $7,000-8,000, a modest sum if the device can be made to measure saturation of carbon monoxide by this completely non-invasive technique.

(6) Reflectance Retinal Densitometry

This approach is based on the spectroscopic measurement of carboxy-hemoglobin or other compounds in blood flowing through vessels in the fundus of the eye. Since blood vessels lie in the outermost layers of the retina, the amount of light scattered may be considerably less than with ear type oximeters. Dr. Paul Brown of Harvard has indicated that reflectance retinal densitometry may be a reasonable way to look at the optical properties of blood to measure specific compounds, e.g., carbon monoxide to estimate smoke intake. This approach obviously requires considerably more study but may represent better access to blood for making in vivo measurements.

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